

**Reproductive biology and controlled pollination of
Eucalyptus - a review**

by

Brad Potts and Peter Gore

**School of Plant Science,
University of Tasmania,
Private Bag 55,
Hobart, Tasmania**

February 28th, 1995

Table of contents

1. The genus <i>Eucalyptus</i>	3
2. Floral morphology and its taxonomic significance	6
Inflorescence.....	6
Flower buds and flowers	8
Operculum.....	10
Stamens	12
Style and Stigma.....	13
Ovary.....	14
Ovule.....	15
Fruit (capsules).....	17
Seed.....	17
Germinates.....	20
Summary of major subgeneric differences.....	22
3. Reproductive Biology	23
Age to first flowering.....	23
Bud initiation and development	24
Flowering time	25
Post-anthesis development	27
Pollination.....	28
Pollen shed	28
Pollen physiology	29
Pollen germination and pollen tube growth	30
Stigma receptivity.....	32
Fertilisation and ovule development	32
Capsule maturation, dehiscence and seed shed.....	33
4. Breeding system	34
Pollen vectors.....	34
Pollen dispersal.....	34
Seed Dispersal	35
Karyotype.....	36
Levels of outcrossing	37
Self fertility	37
Mechanism of self-incompatibility	38
Male sterility.....	39
Effects of inbreeding	39
5. Interspecific hybridisation.....	42
Natural hybridisation.....	42
Artificial hybridisation	43
Barriers to hybridisation.....	45
Structural.....	46
Physiological barriers (incongruity)	47
6. Techniques for controlled pollination	50
Manipulation of pollen.....	50
Flower bud collection.....	50
Dry Pollen extraction	50
Wet extraction	51
Pollen storage.....	52
Testing pollen viability.....	52
<i>In vitro</i> techniques.....	52
Liquid medium.....	52
Agar medium.....	53
<i>In vivo</i> techniques	54
Controlled pollination.....	55
Emasculation.....	55
Isolating flowers	55
Celluloid bags	56
Terylene bags	57
Style isolation.....	57
Post-anthesis floral development	57
Timing of receptivity	57
Morphological changes.....	57
<i>In vivo</i> pollen germination.....	58
Pollination techniques.....	58
Self pollinations	59
Debagging	59
Collection of fruit and seed extraction.....	59
Example record sheet for controlled pollinations	61
Bibliography	62

1. The genus *Eucalyptus*

The genus *Eucalyptus* belongs to the predominantly southern hemisphere family Myrtaceae. The family comprises approximately 155 genera and over 3000 species. Approximately 75 of the genera occur in Australia, predominantly in the wetter regions (Chippendale 1988). Traditionally the eucalypts have included one large genus *Eucalyptus* and a smaller genus *Angophora* (Pryor and Johnson 1981; Chippendale 1988). However, there is considerable debate concerning the taxonomic treatment of *Eucalyptus* and *Angophora* (see Ladiges and Humphries 1983; Sale *et al.* 1993). Johnson and Briggs (1983) have partitioned the subfamily Leptospermoideae into seven alliances, including the *Eucalyptus* alliance (Fig. 1.1) which is believed to be monophyletic with the *Eucalyptopsis* alliance. The latter alliance includes the distinctive genus *Arillastrum* (Johnson and Briggs 1983) which occurs in New Caledonia.

The genus *Eucalyptus* contains over 500 species, most of which are endemic to Australia. Six Australian eucalypt species extend to New Guinea with a seventh endemic there. Three species extend beyond the Australian and New Guinean mainland (*E. alba*, *E. urophylla* and *E. deglupta*) to parts of Malesia and the Philippines. Its distribution appears to follow the full extent of the Australian tectonic plate during the late Tertiary (Barlow 1981; Fig. 1.1) but does not cross Huxley's Line (Eldridge *et al.* 1993). Barlow (1981) considers that the genus is of ancient Australian origin, although it does not appear in the fossil record in Australia until the Miocene (Holmes *et al.* 1982; Hill 1994). However, there are reports of fossils with affinities to *Eucalyptus* from Miocene deposits in both South America (Frenguelli 1953) and New Zealand (Pole 1989) and there is the possibility that major lineages had differentiated by the mid-Miocene (Holmes *et al.* 1982). If correct, these fossils indicate a wider past distribution and possibly a more ancient, Gondwanic origin of the genus. Nevertheless, the dramatic increase in *Eucalyptus* pollen in Pleistocene and more recent deposits (Singh *et al.* 1981) suggests that the genus has recently undertaken a rapid spatial, and possibly major evolutionary radiation in the last 200,000 years as a response to increasing aridity, more seasonal climates and an increase in the occurrence of fire in Australia. They currently dominate nearly all vegetation types in Australia except rainforest and allied mesic types and only in the arid interior of Australia are eucalypts generally lacking in dominance.

The genus *Eucalyptus* is divided into 8 subgenera - *Blakella*, *Corymbia*, *Eudesmia*, *Symphyomyrtus*, *Idiogenes*, *Gaubaea*, *Monocalyptus* and *Telocalyptus* (Pryor and Johnson 1971; Pryor and Johnson 1981; Ladiges and Humphries 1983), although (Johnson and Briggs 1983) treats them as having equal rank to *Angophora*. The validity of the eight subgenus, *Telocalyptus*, described by Johnson (1976), has recently been questioned (Drinnan and Ladiges 1991b; Sale *et al.* 1993).

The distribution of the subgenera are shown in Fig. 1.2. The subgenus *Blakella* is composed of 7 species, commonly called the 'paper-fruited bloodwoods', which are distributed in the northern half of Australia and several species are indigenous to New Guinea. The subgenus *Corymbia* comprises about 35 species which are commonly referred to as the 'woody-fruited bloodwoods' are widely distributed mainly in the northern part of Australia and the southwest of Western Australia. Both *Corymbia* and *Blakella*, have affinities to *Angophora*, and have certain primitive characteristics which place them apart from the other subgenera of *Eucalyptus* (Boland, *et al.* 1985). The *Eudesmia* comprise about 16 species which (Ladiges and Humphries 1983) treat as four subgroups due to the atypical characteristics of several species (*E. gamophylla*, *E. baileyana* and *E. similis*). The *Monocalyptus* consists of about 100 species including the world's tallest hardwood species (*E. regnans*; height 96m). This subgenus is confined to the wetter areas of south west,

southern and eastern Australia and comprises two major sections *Hesperia* (confined to Western Australia) and *Renantheria* (mainly in eastern Australia). In general the *Monocalyptus* species have not performed well in exotic plantings outside of Australia (Pryor 1976), although within Australia, several of the ash species from this subgenus are of commercial value in forestry (e.g. *E. marginata*; *E. regnans*). The two minor subgenera *Ideogenes* (1 species *E. cloeziana*) and *Gaubaea* (2 species *E. curtisii* and *E. tenuipes*) have close phylogenetic affinities to the *Monocalyptus* (Ladiges and Humphries 1983; Sale *et al.* 1993). The subgenus *Symphyomyrtus* contains the majority of species in the genus (over 300 sp.) and is widely distributed throughout Australia. It comprises 9 or 10 sections with numerous series and contains most of the eucalypt species utilised commercially throughout the world (e.g. *E. grandis*, *E. urophylla*, *E. globulus*, *E. nitens*, *E. camaldulensis* and *E. viminalis*). The subgenus *Telocalyptus* is a minor subgenus (Johnson 1976) includes four tropical species *E. howittiana*, *E. brachyandra*, *E. raveretiana* and *E. deglupta*. However, this may not be a natural group with one of the species at least, *E. deglupta*, having closer affinities to the *Symphyomyrtus* (Sale *et al.* 1993).

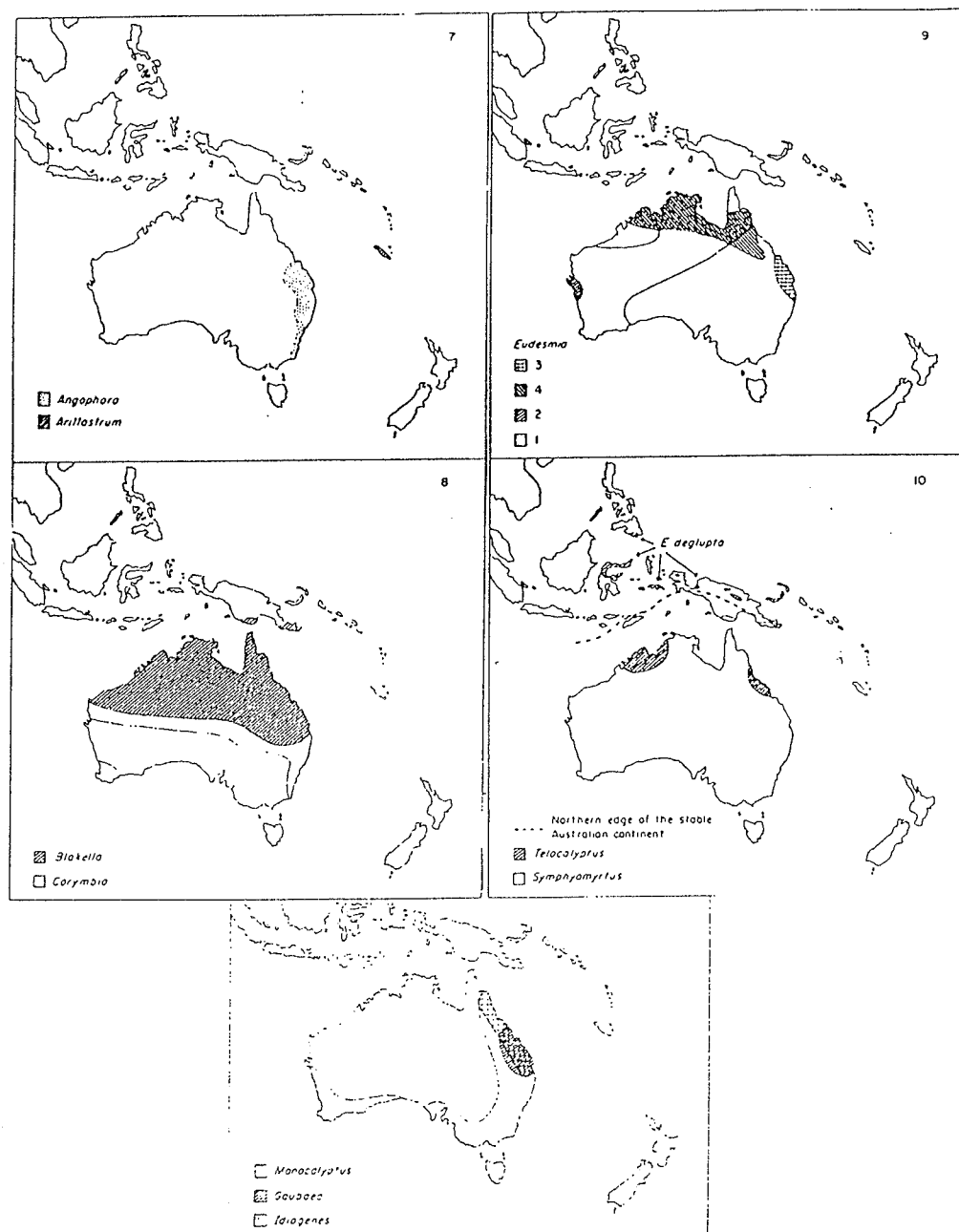


Fig. 1.1 The distribution of *Arillastrum*, *Angophora* and the subgenera of *Eucalyptus* (from Ladiges *et al.* 1983).

SUBGENUS	<i>BLAKELLA</i>	<i>CORYMBIA</i>	<i>MONOCALYPTUS</i>	<i>SYMPHYOMYRTUS</i> *						
SECTION				<i>Transversaria</i>	<i>Bueclaria</i>	<i>Dumaria</i>	<i>Exsertaria</i>	<i>Maidenaria</i>	<i>Adnataria</i>	<i>Sebaria</i>
VERNACULAR NAMES	Ghost Gums	Bloodwoods	Stringybarks Peppermints Ashes	Flooded Gum Red Mahogany Grey Gums	Mallees	Mallees	Red Gums	Gums	Boxes Ironbarks	Tallow Wood

Fig. 1.2 The principal subgeneric taxa in the genus *Eucalyptus* (from Pryor 1976).

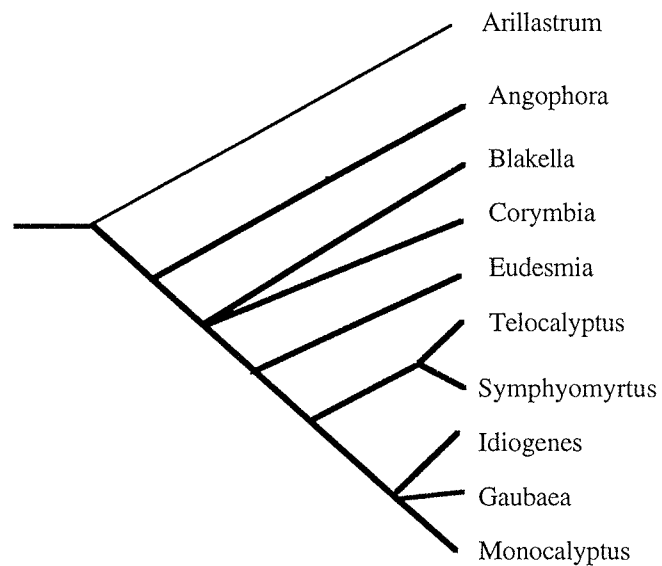


Fig. 1.3 The phylogenetic relationship amongst the *Eucalyptus* subgenera (modified from Ladiges and Humphries 1983, p.119)

2. Floral morphology and its taxonomic significance

Inflorescence

Eucalypt flowers generally occur in clusters in an inflorescence which is borne in the axil of a leaf. The individual flower buds form singly or more generally in units of 3, 7, 9, 11, 15 etc. (Fig. 2.1). Single flowers in the axil of a leaf are found in only a few species (e.g. *E. globulus*).

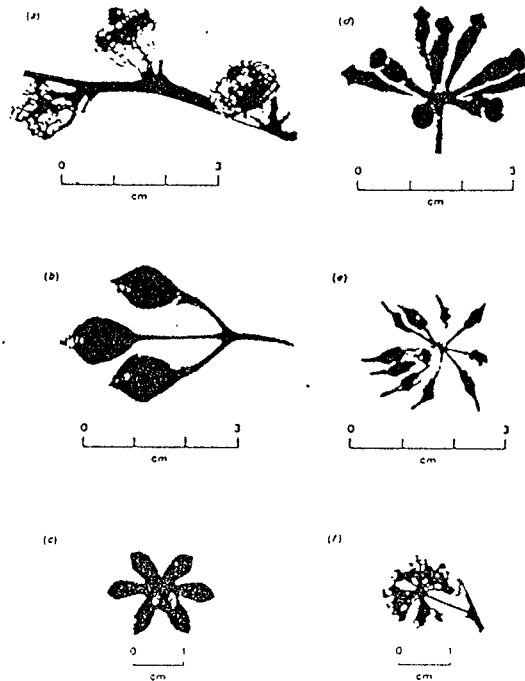


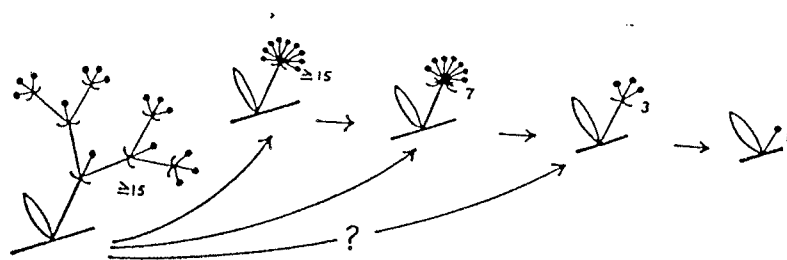
Fig. 2.1 A range of flower bud numbers per unit inflorescence (umbel) in *Eucalyptus*. (a) 1 *E. globulus* (b) 3 *E. longifolia* (c) 7 *E. goniocalyx* (d) 9 *E. cladocalyx* (e) 11 *E. socialis* (f) >11 *E. amygdalina* (from Boland, et al. 1985).

In eucalypts there are various degrees of aggregation, compaction and expansion of the total inflorescence (Pryor and Johnson 1971). The unit inflorescence is generally called an 'umbel' or 'umbellaster' but is actually a condensed dichasium in which the intermediate axes are totally reduced and some branches are absent (Fig. 2.2). Each unit inflorescence is characteristically located in a leaf axil and attached by a peduncle. The total number of buds per inflorescence is frequently of importance at lower taxonomic levels.

Flower clusters may arise singly in the leaf axils (simple axillary inflorescence), as paired axillary inflorescences, or compound axillary or terminal inflorescences (Fig. 2.3). The axillary unit inflorescence (umbel) is universal in the *Monocalyptus* where it has undergone little secondary change. It is the most usual condition in the *Eudesmia* and widespread in the *Symphyomyrtus*. The compound inflorescences are developed in a manner which is quite unique to eucalypts (Pryor 1976). They are composed of unit

inflorescences which are attached to axils at which the leaf has been reduced or nearly completely suppressed but the associated node persists in the alternate arrangement. The peduncle of each unit inflorescence is

(a) Unit inflorescence



(b) Compound inflorescence

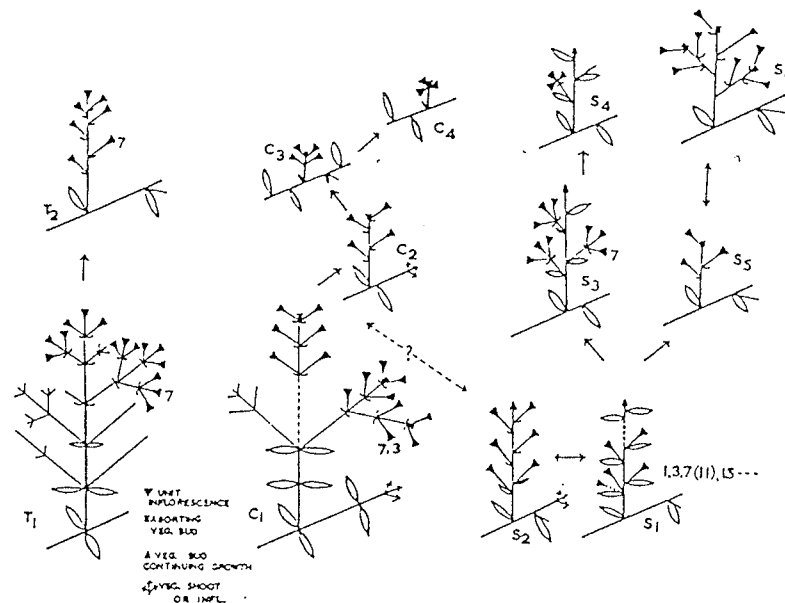


Fig. 2.2 Derivation of the eucalypt (a) unit inflorescences from an indefinite dichasium (b) and compound inflorescence (from Johnson 1972).

attached to the reduced leaf axil and unit inflorescences are aggregated into compound groups by shortening internodes and intranodes. This results in a structure resembling the classical panicle which Pryor (1976) terms a 'eucalyptoid compound inflorescence'. Compound inflorescences occur in *Angophora*, *Blakella*, *Corymbia* and *Gaubaea* although structures superficially resembling these compound inflorescences, but of different derivation, may be found in the other subgenera.

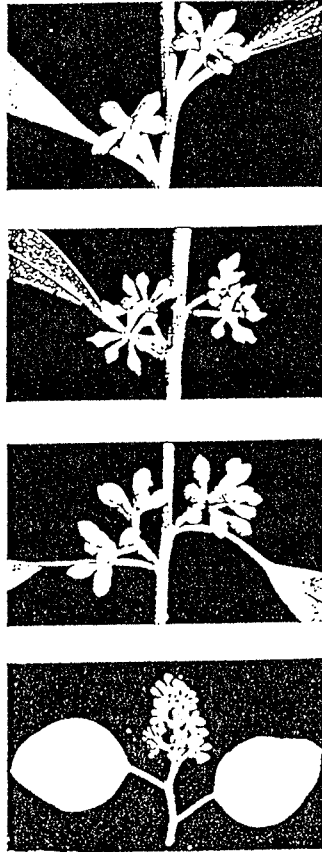


Fig. 2.3 A range of inflorescence types found in eucalypts. Simple axillary inflorescence, *E. pauciflora* (top), paired axillary inflorescences, *E. fastigata*, compound axillary inflorescences, *E. michaeliana*, and a compound terminal inflorescence *E. polyanthemos* (bottom) (Brooker and Kleinig 1983).

Flower buds and flowers

The flower buds initially develop enclosed in a bract which is shed as the buds swell (Fig. 2.4). There is much variation in the size and shape of the flower buds, although within a species this tends to be relatively stable. The flower bud consists of a stalked (pedicelate) or sessile hypanthium (a hollow receptacle) which is covered by one or two cap-like opercula which protect the male and female parts of the flower but is shed at anthesis (Fig. 2.5). It is customary to refer to the flower as a 'bud' until the stage of opercula shed after which it is referred to as the 'flower'.

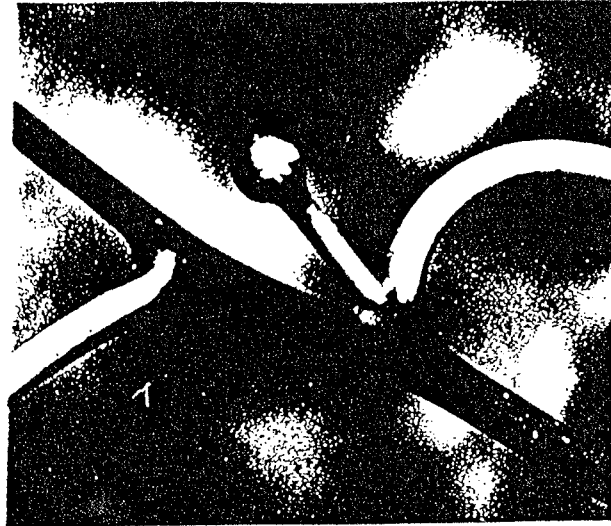


Fig. 2.4 Inflorescence of *E. nitens* enclosed by floral bracts (photo P. Volker).

The eucalypt flower is normally bisexual and has some of the general Myrtaceous characteristics such as the large numbers of stamens and the perigynous arrangement of the floral whorls (Fig. 2.5). The ovary contains 2-7 locules, each containing numerous ovules. At anthesis, the operculum or opercula are shed and the stamens spread resulting in a conspicuous floral display. The colour of the eucalypt flower is determined by the colour of the filaments. Filaments of most species from the east of Australia are generally white whereas species from the west of Australia often have brightly coloured filaments (Griffin 1982). The ovary which contains the placentae and ovules is embedded either partially or completely within the hypanthium (receptacle) while the style is emergent and visible (Fig. 2.7). The numerous stamens are usually arranged in whorls, their point of attachment to the flower being termed the staminal ring (or staminophore). There are no definite nectaries formed in eucalypts and nectar production appears to be a function of the epidermal cells lining the floral cup between the staminal ring and the base of the style (Davis 1969).

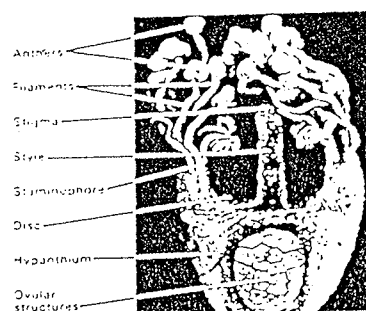
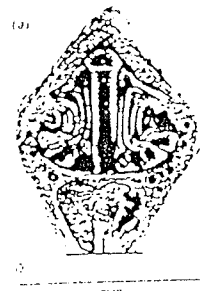


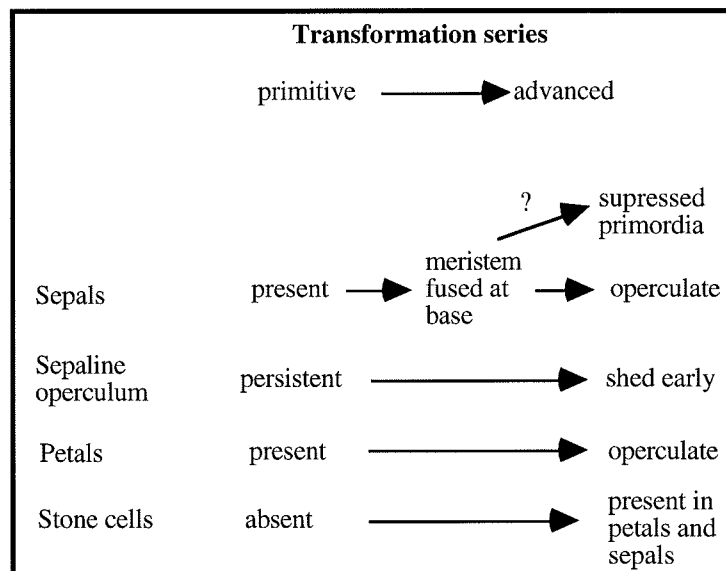
Fig. 2.5 Cross section through flower buds showing anthers (a) enclosed by the operculum and (b) after the operculum has been shed. (Brooker and Kleinig 1983)



Operculum

The presence of the operculum is one of the most distinctive features of *Eucalyptus* (Pryor 1976). The operculum is derived from fusion of the petals or sepals. The closely related genus *Angophora* does not have an operculum and the sepals and petals are free. There is considerable variation in the genus in the actual anatomical structure of the operculum and this is one of the key traits used to differentiate the *Monocalyptus* and *Symphyomyrtus* subgenera (Fig. 2.6).

The *Symphyomyrtus* species have two opercula, the inner derived from the fusion of the petals, the outer operculum derived from the fusion of the sepals. These two opercula may be free from each other in which case the outer operculum may be shed early during bud development, fully joined or fragmented. In other cases, both the sepaline and petaline opercula may fuse and be shed together at anthesis. In some species four unfused sepals occur around the rim of the hypanthium appearing as small teeth-like structure on the top of the hypanthium or they may occur toward the top of the inner operculum (Fig. 2.6). The *Monocalyptus* species have only a single operculum and it has been difficult to determine whether it is derived from the sepals or petals or even a combination of both (Pryor 1976). Pryor and Knox (1971) considered that the single operculum in the *Monocalyptus* was derived from the fusion of the sepals (sepaline). However, this is questioned by Drinnan and Ladiges (1989) who consider that it is more consistent with trends in closely related groups for the single operculum to have originated from fused petals.



When two opercula are originally present and the outer operculum is shed during bud development, a ring scar (prominent or obscure) is carried on the bud for the rest of its development. If the outer operculum is carried until flowering (either separate or fused with the inner operculum), the side of the bud is smooth. Where only one operculum is present from the beginning, no scar occurs although towards flowering, a ring of dying tissue at the junction of the operculum and the hypanthium may have the appearance of a scar (Brooker and Kleinig 1983).

SUBGENUS	FLOWER BUD: YOUNG, ANTHESIS	ANTHER	COTYLEDONS	INFLO.	OVULE	STIGMA TYPES
[GENERALIZED ANCESTOR]			?	DICHASIAL THYRSE		mop uniform
ANGOPHORA				C ₁	h	mop uniform
BLAKELLA				C ₂ , C ₃ , C ₄	h	tapered lobed
CORYMBIA				C ₁ C ₂	h	mop uniform
EUDESMIA				S ₁ , S ₂ , S ₄	h	blunt lobed
GAUBAEA				C ₁ , C ₂	a	-
IDIOGENES				T ₂	a	-
MONOCALYPTUS				S ₁	a	blunt hollow
SYMPHYOMYRTUS				T ₁ (SB, SS) S ₁ (SE, SI, SL, SM, SP, SU) S ₂ (SO) S ₃ (SH) S ₅ (SU, SW) S ₆ (SV)	h	blunt lobed

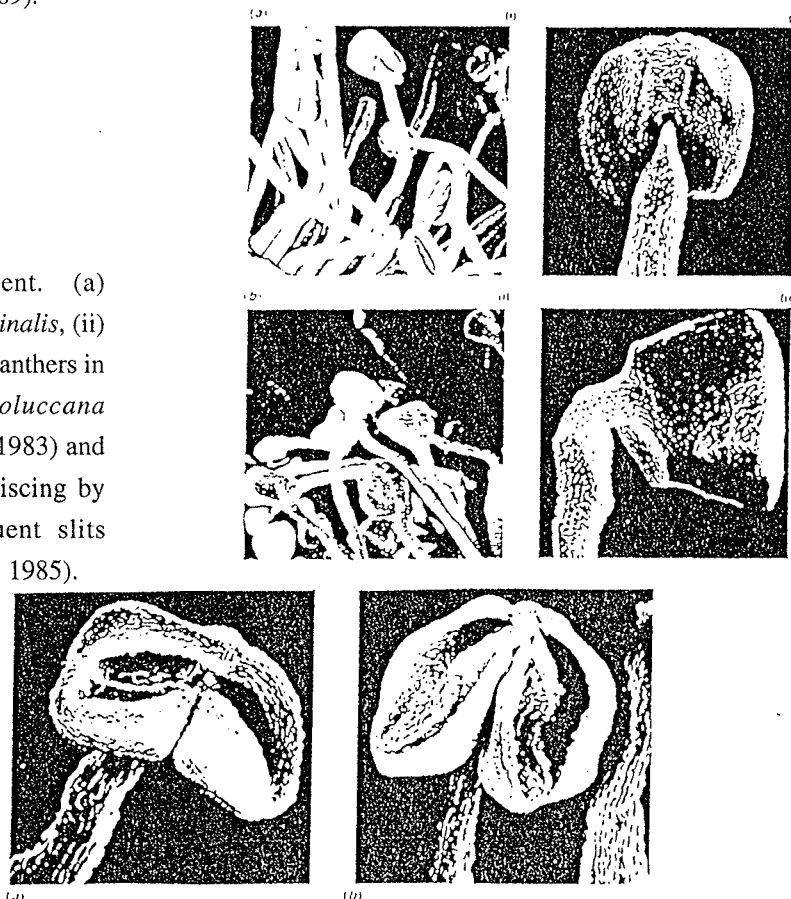
Fig. 2.6 The characteristic operculum, anther, inflorescence, ovule and stigma types of the *Eucalyptus* subgenera (from Johnson 1972) stigma information from Boland and Sedgley (1986). Codes for the compound inflorescences are given in Fig. 2.2. Ovule types correspond to hemitropous (h) and anatropous (a).

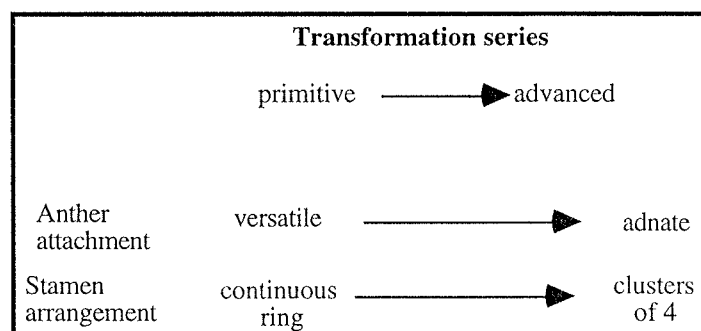
Stamens

The stamen comprises both a filament and anther. The filaments may vary in the manner in which they are compacted when enclosed by the operculum prior to expansion when the opercula are shed (inflexed, flexuose and erect). In some species the outer filaments are infertile, having no anther or a reduced non-functional anther at their summit. These sterile stamens are termed 'staminodes'. The staminode to anther ratio may differ markedly between species. For example, Moncur and Boland (1989) found no staminodes in the three *Monocalyptus* species but nearly one third of the filaments were staminodes in the three *Symphyomyrtus* species examined. The length of the stamen increases from the inner (shortest) to the outer (longest) whorls on the staminophore (Moncur and Boland 1989). The anthers consist of two sacs set on opposite sides of a longitudinal connecting tissue (the connective). Anthers may be attached to the filament at the base of the connective tissue or at the back. The anthers may either pivot freely at the top of the tapered filament (versatile), or be attached firmly to the top of a non-tapering filament (adnate) (Fig. 2.7).

There are numerous forms of anthers (Blakely 1934; Fig. 2.6). The versatile anthers with long, separate dehiscence-slits are wide spread in the genus (*E. camaldulensis*, *E. gunnii*, *E. viminalis*, *E. globulus* etc.) but may be shortened in various series. A kidney shaped anther in which the two sacs dehiscence by oblique joined (confluent) slits occurs in the *Monocalyptus* section *Renantherae* whereas a small group of species from the subgenus *Monocalyptus* in Western Australian (section *Hesperia*) have the more general, non-confluent anther shape (Boland *et al.* 1985; Ladiges and Humphries 1986). It appears that the confluent, kidney shape anther has evolved after the divergence of the subgenus *Monocalyptus* (Johnson 1972). Most diversity in anther type occurs within the subgenus *Symphyomyrtus* (Fig. 2.6) where completely adnate anthers with more or less pore-like opening are characteristic of the section *Adnataria*. An anther gland is often found at the apex of the anther, close to the point of attachment of the filaments and consists of a circular cavity lined by glandular cells (Davis 1969).

Fig. 2.7 Anther attachment. (a) versatile anthers in (i) *E. viminalis*, (ii) *E. planchoniana*, (b) adnate anthers in (i) *E. foecunda*, (ii) *E. moluccana* (from Brooker and Kleinig 1983) and *Monocalyptus* anthers dehiscing by confluent and non-confluent slits (bottom) (from Boland *et al.* 1985).





Style and Stigma

The eucalypt style is simple, but differs in length, width and rigidity between species. The eucalypt stigma is a wet stigma (following Heslop-Harrison and Shivanna 1977) as a sticky mucilageneous secretion covers the stigmatic surface at receptivity (Anderson 1984). Stigma and style morphology vary greatly but are related to the taxonomic groupings (Figs. 2.6 and 2.8 ; Boland and Sedgley 1986). All species have papillate stigmas

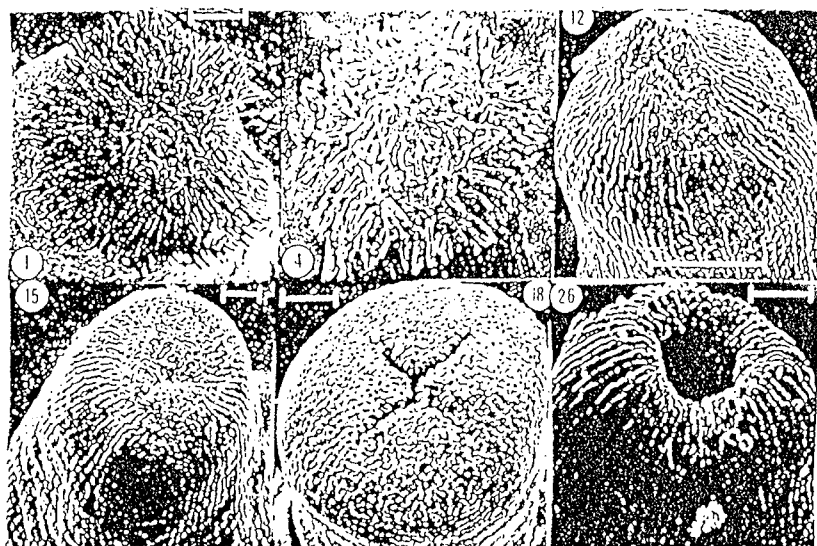
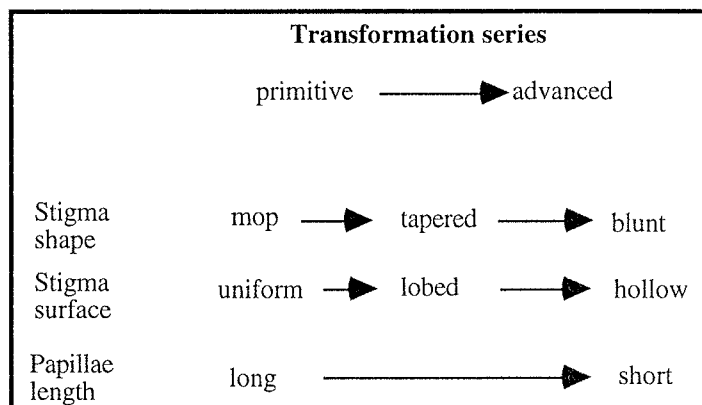


Fig. 2.8 Representative stigma types for the major eucalypt subgenera. The mop, uniform type style of *Angophora* (1) and subgenus *Corymbia* (4), the tapered lobed style typical of the subgenus *Blakella* (12), the blunt lobed styles typical of the subgenera *Eudesmia* (15) and *Symphyomyrtus* (18) and the blunt, hollow style typical of the subgenus *Monocalyptus* (26) (Boland and Sedgley 1986).

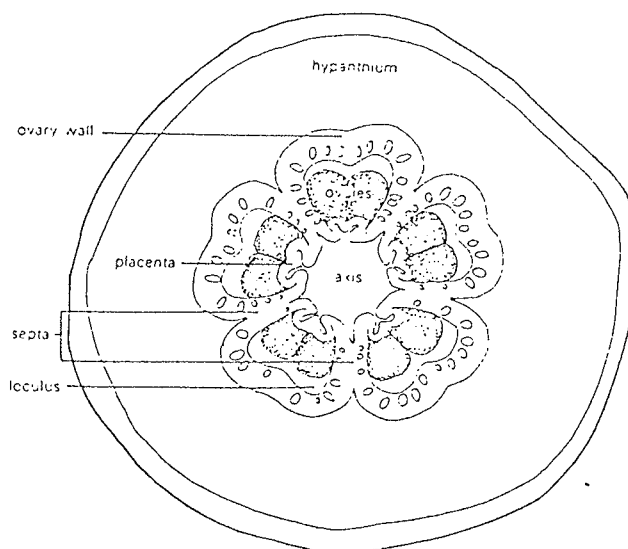
and a styler canal of varying length (Boland and Sedgley 1986). Species from the genus *Angophora* have mop-like stigmas with long papillae which are very similar in appearance to the red bloodwood group of the subgenus *Corymbia* (bloodwoods). The spotted gum group of the *Corymbia* have mop-like stigmas with short papillae whereas the yellow bloodwoods have tapered stigmas. All of the *Blakella* species have tapered stigmas with a lobed surface and relatively short papillae. The *Eudesmia* are relatively variable. Most *Symphomyrtus* species have blunt or pin-head shaped stigmas with a heavily cutinised styler canal whereas *Monocalyptus* species have blunt stigmas with few papillae and hollow styles (Boland and Sedgley 1986).



Ovary

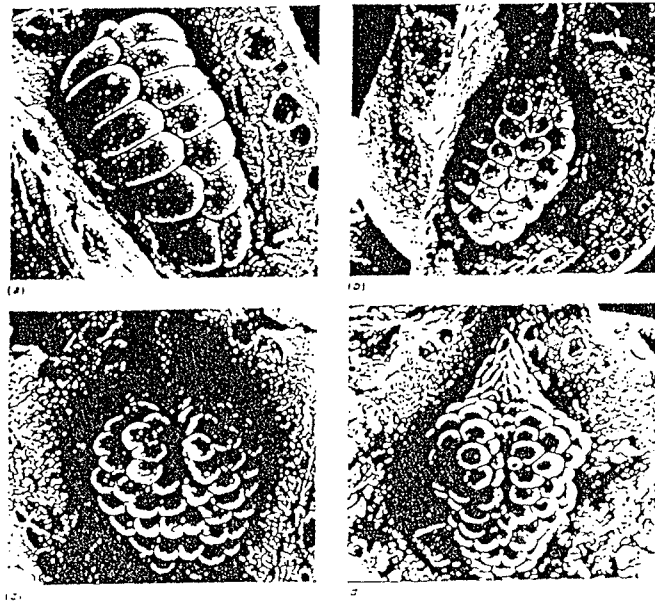
The eucalypt ovary is multi-locular with many ovules per locule (Fig. 2.9), but the number of each varies between species. The locules (or chambers) are joined to a common central axis. The placentae bear ovular structures of different types. Those toward the base of the placenta are the last initiated, whereas those towards the top are the first to be initiated and comprise congenitally sterile structures, termed ovulodes (Carr and Carr 1962). The unfertilised ovary of *E. regnans* contains a mean of 31 ovular structures comprising 16.5 normal ovules, 2.5 abnormal ovules and 11.5 sterile ovulodes (Sedgley *et al.* 1989). In contrast, the ovary of *E. woodwardii* contained a mean of 280 ovular structures, of which 79 were ovulodes, 160 were fertile ovules, and 41 were abnormal (Sedgley 1989).

Fig. 2.9 Cross section through the ovary of *E. preissiana* (from Boland *et al.* 1980).



The number of locules, ovules and ovulodes and their placement on the placenta is of taxonomic significance (Carr and Carr 1962). The number of longitudinal rows in the lower in the lower part of the placenta is two in the *Monocalyptus*, except in snow gums such as *E. pauciflora* and *E. coccifera*, where there are three or four rows. The *Eudesmia* also tend to have ovules in two rows although these often tend to be widely separated exposing the placenta, a condition not found in the *Monocalyptus* (Boland *et al.* 1980). The remainder of *Eucalyptus* and *Angophora* have ovules which are either not in distinct rows or are clearly in rows of 4, 6, 8 or 10 (Ladiges and Humphries 1983; Fig. 2.10).

Fig. 2.10 The different arrangement of ovules in the ovaries of various *Eucalyptus* species (from Boland, et al. 1985). (a) Two rows *E. pauciflora*, (b) four rows *E. flocktoniae*; (c) six rows *E. blakelyi*; (d) eight rows *E. pellita*.



Ovule

The ovule is the structure in which meiosis and megaspore formation takes place. The typical angiosperm ovule is shown in Fig. 2.11. In most angiosperms the mature female gametophyte consists of seven cells with eight nuclei (Fig. 2.11). The outermost layers of the ovule generally form two integuments, which partly envelope the central mass of tissue, termed the nucellus, and later form the seed coat (testa). The pollen tubes enter the embryo sac through the micropyle which is formed by the free ends of the integuments. Each ovule must be fertilised by one separate pollen tube. However, multiple paternity may exist within a capsule. Rare ovules with more than one embryo sac have also been observed in *Eucalyptus* and it is possible that they may account for observations of seed with multiple embryos (Sedgley 1989).

Johnson and Briggs (1983) separates the major suballiances in *Eucalyptus* on the basis of the orientation of the ovule. Most of the eucalypts (e.g. *Symphyomyrtus*) have ovules which are orientated away from the placenta (point of attachment) (hemitropous or campylotropous) (Figs. 2.6 and 2.12) whereas the *Monocalyptus* suballiance have anatropous ovules in which the micropyle faces the placenta as shown in Fig. 2.12. The *Monocalyptus* (including *Idiogenes* and *Gaubaea*) have homologous ovule, and seed characters (Ladiges and Humphries 1983). The anatropous condition, where the ovule becomes completely inverted and the micropyle faces the placenta is common in the *Myrtaceae* but hemitropous and campylotropous ovules do

occur. *Angophora* has hemitropous ovules and Ladiges and Humphries (1983) considers the anatropous condition as the most derived condition.

Fig. 2.11 The typical angiosperm anatropous ovule showing funicle (f), vascular bundle (vb), chalaza (c), outer integument (oi), inner integument (ii) forming the micropyle (m), nucellus (n) and embryo sac (es). The embryo sac is not to scale and contains two synergid cells (s) with filiform apparatus (fa), an egg cell (e), three antipodals (a) and a polar fusion nucleus (pfn) (from Sedgley and Griffin 1989).

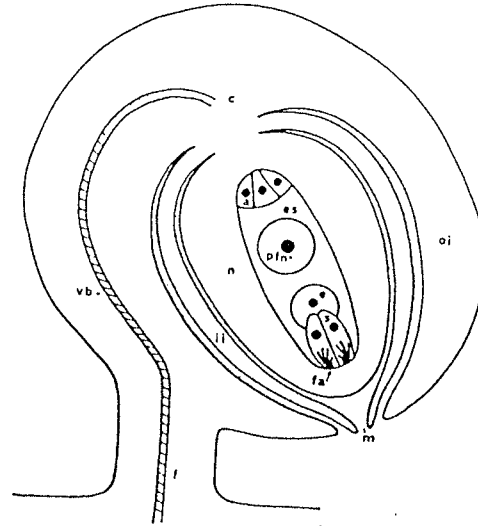
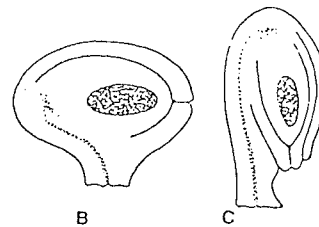
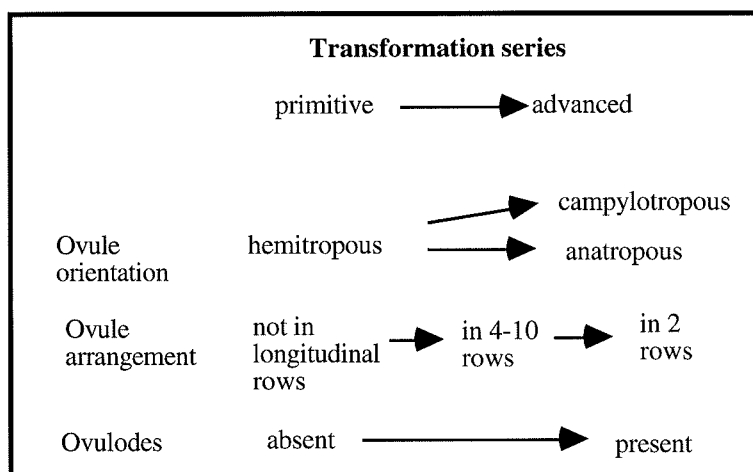


Fig. 2.12 Sectional views of hemitropous (B) and anatropous (C) ovules (from Foster and Gifford 1974).



There have been few detailed studies of the structure of the embryo and ovule in eucalypts from which general trends can be detected. In the few cases examined (e.g. Davis 1968, 1969; Sedgley 1989; Sedgley *et al.* 1989) it appears that the antipodal cells are absent from the embryo sac at maturity, the cytoplasm of the egg cell is scant, and that the time of fusion of the polar nuclei and the site of the occurrence of sterile ovules are variable. However, the ovulodes (Carr and Carr 1962) are a quite distinct category of sterile ovular structure

in which the development is arrested at an early stage (Sedgley 1989), and no penetration of these structures was ever observed by Sedgley *et al.* (1989). The structure of the ovulodes appears to differ between species although they are mainly located at the stylar end of the locule. In *E. woodwardii*, the ovulodes consisted of outer and inner integument only (Sedgley *et al.* 1989), the nucellus and a single integument in *E. stellulata* (Davis 1969), and outer integument with only occasional inner integument development in *E. regnans*. The development of ovulodes appears to be a derived trait (Ladiges and Humphries 1983). Their functional significance appears unclear, although Sedgley *et al.* (1989) suggests that they may serve to protect fertile seed from insect predation and infection. These structures are quite distinct from abnormal ovules which cease development at a much later stage and show no positional pattern on the placenta (Sedgley *et al.* 1989).



Fruit (capsules)

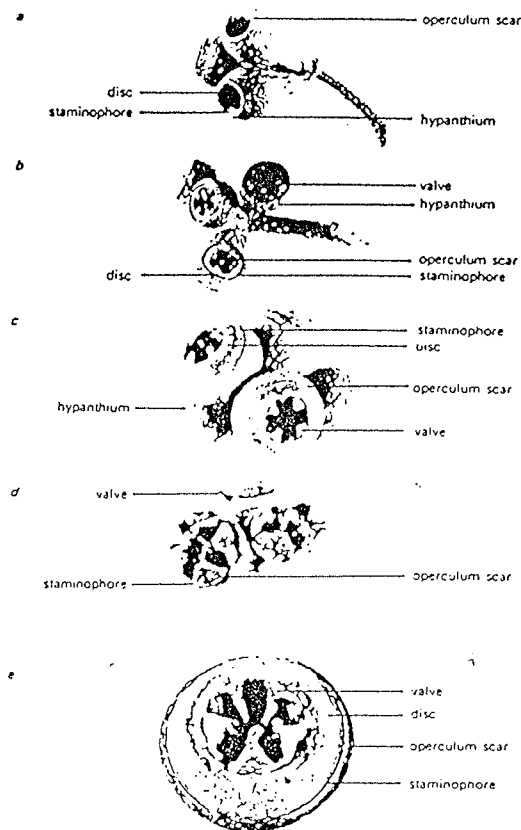
The eucalypt capsule is a false fruit being developed from the inferior ovary which is intimately fused to the hypanthium (Cremer 1965b). After fertilisation, the ovary matures inside the receptacle and forms a woody dehiscent capsule which on drying will liberate seeds through openings formed by the spreading of valves on the top. The valves are derived from the splitting of the ovary roof into sectors corresponding to the locules (Boland *et al.* 1985). Several distinctive rings are often evident on the capsule. The outer most is the operculum scar and the inner is the scar of the staminal ring. Within these bands is the disc which is derived from the nectary of the flower (Boland *et al.* 1980; Fig. 2.13). Capsule size can vary considerably both within a tree with the stage of development and between trees and seasons (Ladiges and Ashton 1974), but capsule shape in particular is relatively stable and frequently of taxonomic importance.

Seed

Eucalypts produce seed with no endosperm and the newly emerged seedling is sustained by the photosynthetic cotyledons (Boland *et al.* 1980). As in other angiosperms with endosperm-less seed, the endosperm is probably absorbed by the developing embryo. The embryo is contained within a seed coat (testa) derived from the ovule integuments which undergo changes of structure, hardness and colour during maturation. The point of former attachment of the ovule to the placenta is evident as a scar on the seed (hilum). The hilum may be ventral or terminal (depending on the ovule orientation), conspicuous or inconspicuous, white or coloured (Boland *et al.* 1980).

Eucalyptus has undergone considerable evolutionary divergence with respect to seed characteristics such as anatomical structure, shape and testa colour and patterning (Boland *et al.* 1980). Seed with two integuments is common in the Myrtaceae and occurs in *Arillastrum*, *Angophora* and most of the subgenera of *Eucalyptus* (Ladiges and Humphries 1983). However, in the subgenera *Teleocalyptus* and *Symphyomyrtus*, there is only one integument, and the inner is believed to be resorbed during development (Gauba and Pryor 1958). The outer integument is only two cells thick in the *Symphyomyrtus* and *Teleocalyptus* and this is believed to be a derived state (Ladiges and Humphries 1983).

Fig. 2.13 Fruit of several species of *Eucalyptus* showing discs of varying form (from Boland *et al.* 1980).



The eucalypt capsule contains fertile seed, aborted seed and chaff (Drake 1975; Boland *et al.* 1980), all of which is normally found in a seedlot. The chaff is derived from the non-functional ovulodes towards the top of the ovary as well as abnormal or unpenetrated ovules which have aborted early in their development. In some *Symphyomyrtus* species two types of chaff are produced (Boland *et al.* 1980; Fig. 2.14). The first type are elongated particles derived from the upper part of the placenta where it is incurved and supports elongated, more or less erect, ovulodes. The second type of chaff consisted of more cubical particles derived from lower down the placenta and could include congenitally sterile ovulodes from inner rows (e.g. Fig. 2.14),

abnormally developed ovules and unpenetrated normal ovules (Sedgley 1989). In contrast, *Monocalyptus* species produce fewer and less variable seed structures and the morphological difference between the ovulodes and abnormal or unfertilised ovules is less. Species in this group have only two vertical rows of

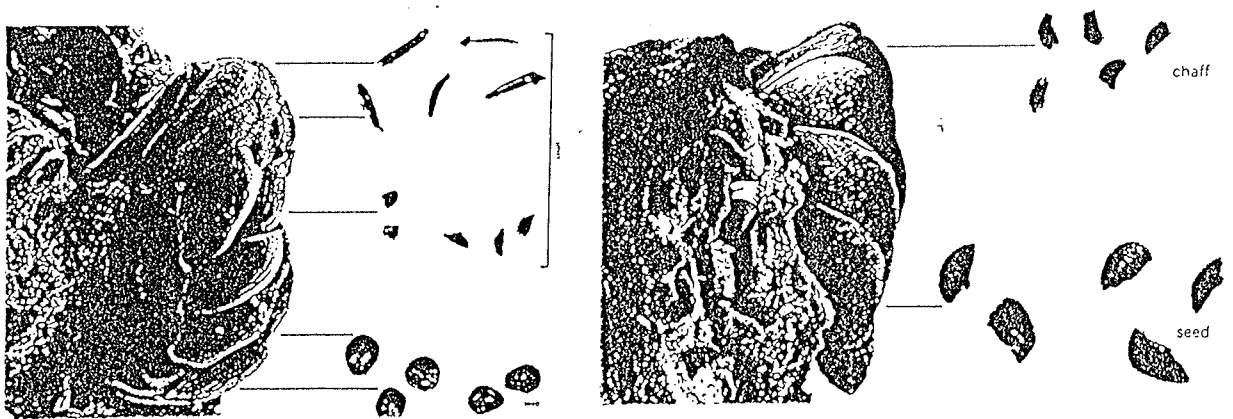
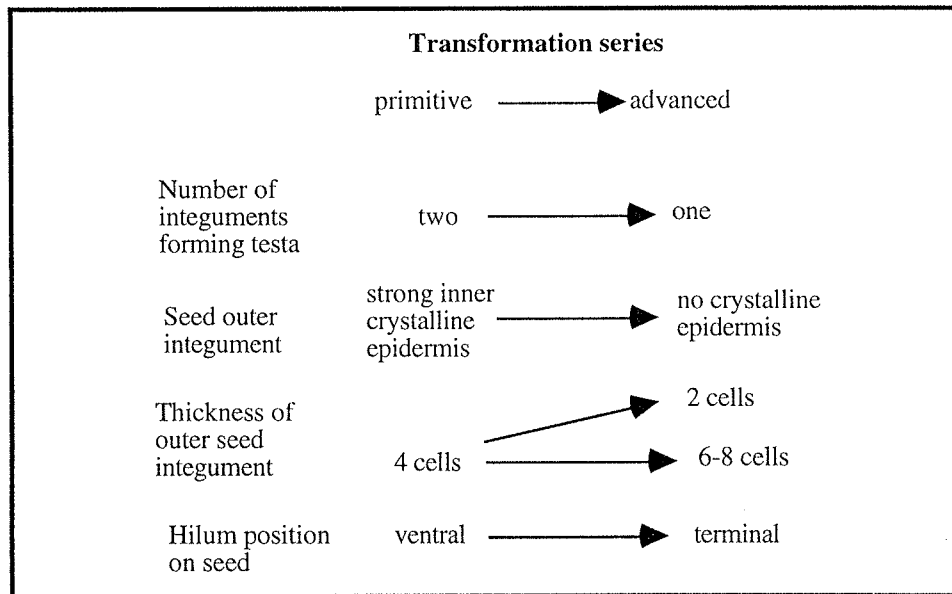


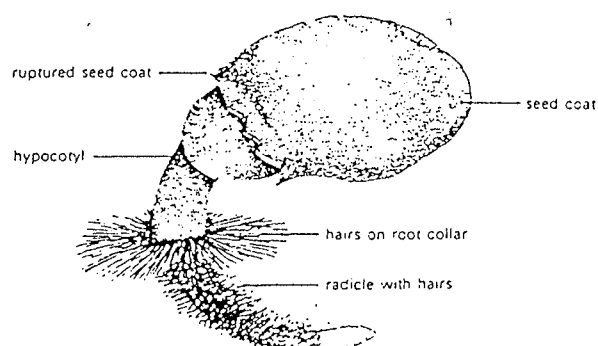
Fig. 2.14 Intact ovular structures on a single placenta and derivatives in a seed lot of (left) *E. alba* (*Symphyomyrtus*) and (right) *E. regnans* (*Monocalyptus*) (from Boland *et al.* 1980).

ovular structures. There are consequently no inner rows and the chaff consists only of material derived from the reduced sterile structures at the top of the placenta plus unfertilized ovules (Boland *et al.* 1980). Sedgley (1989) recognised four types of structures in seedlots of *E. woodwardii* (*Symphyomyrtus*) - full seed, shrunken seed, small seed and ovulodes. The seed categories were differentiated by length and width and only the full seed germinated. The majority of structures classified as small seed were unpenetrated and thus unfertilised ovules in which the nucellus and embryo sac had degenerated by 6 weeks after fertilisation. Structures assigned to shrunken seed appeared to have resulted from the abortion of ovules which lacked endosperm development. On the other hand, Drake (1975) differentiated two types of shrunken seed, both of which were believed to be the results of embryo abortion.

Germinates

The germinating embryo consists of a root, a root collar, a hypocotyl, a pair of cotyledons, an epicotyl (stem between the cotyledons and the first seedling leaves) and an apical meristem (Fig. 2.15). The cotyledons are green and photosynthetic and sustain the emerging plant. They may be green or purple (anthocyanin) on the undersurface (Wilcox 1982)) and range in shape from reniform, bilobed to bisected (Fig. 2.16). The shape of the cotyledon is diagnostic of some groups (Fig. 2.6), with the *Symphyomyrtus* section *Bisectaria* being defined on the basis of the bilobed cotyledons. *Arillastrum* and *Angophora* both have entire cotyledons and this state is believed to be the more primitive (Johnson 1972; Ladiges and Humphries 1983). Taxonomic groups also differ in the manner in which the cotyledons are folded within the testa (see Boland *et al.* 1980).

Fig. 2.15 Germinating eucalypt seed
(from Boland *et al.* 1980)



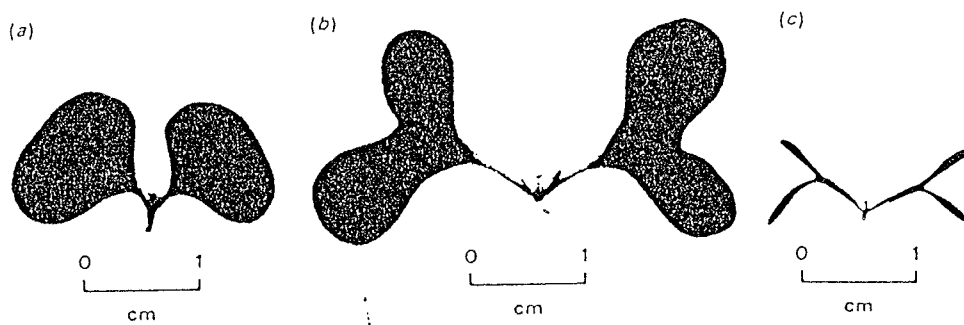
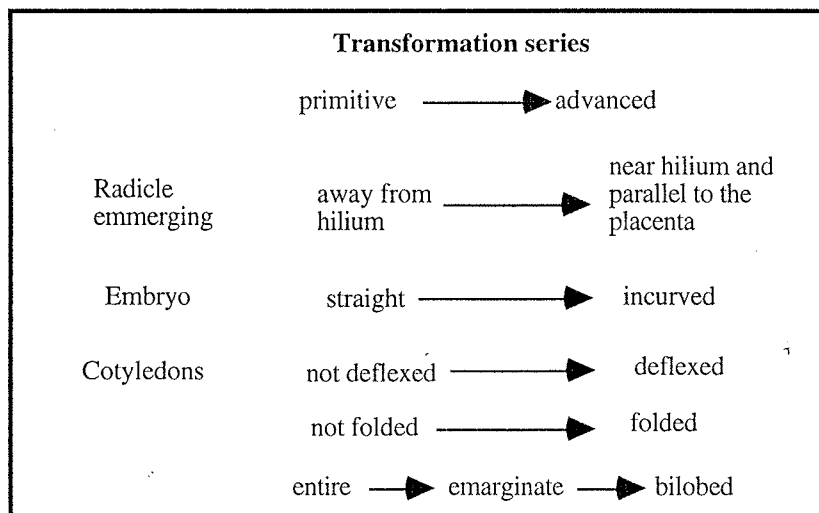


Fig. 2.16 Reniform (a), bilobed(b), and bisected(c) cotylendons of *Eucalyptus*. (from (Boland *et al.* 1985)).



Summary of major subgeneric differences

General differences in reproductive traits between the *Eucalyptus* subgenera and *Angophora* are shown in Fig. 2.6 and summarised below as described by Boland *et al.* (1985).

Angophora

The genus *Angophora* is very closely related to the eucalypt 'bloodwoods'. The flower of this genus does not have an operculum and the petals and sepals are free. The stigma is mop-like and uniform with long papillae and the inflorescence is compound.

***Blakella* (Paper-fruited bloodwoods)**

The subgenera *Blakella* and *Corymbia* have close affinities to the genus *Angophora* and they exhibit primitive characters which place them apart from the other *Eucalyptus* subgenera. The *Blakella* species are differentiated from the *Corymbia* by the thin wall of the mature fruit. The inflorescences are compound and decussate, arranged in umbels on axillary rachis, but the main rachis is often shortened obscuring the true arrangement. Similar inflorescences only occur in *E. cloeziana* (*Ideogenes*) and *E. michaeliana* (*Symphyomyrtus*). The flower buds of the *Blakella* lose their outer operculum early in development unlike most of the *Corymbia*. The style is generally tapered and lobed. Anthers are oblong, versatile and open by longitudinal slits. The fruits mature and shed seed rapidly, sometimes within days of flowering. The seeds are saucer shaped with the cotyledons pressed together unfolded.

***Corymbia* (Woody-fruited bloodwoods)**

The compound inflorescences of the *Corymbia* are large terminal panicles and the anthers are similar to those of the *Blakella*. The stigma are generally mop-like with short papillae and the style are tapered. The *Corymbia* is divided into two sections *Rufaris* and *Ochraria* on the basis of the presence of winged seed in most of the section *Rufaris* and the early loss of the operculum in the section *Ochraria*.

Eudesmia

Most species have simple axillary (unit) inflorescences with the bud numbers varying from 3 to numerous and the anthers similar to the those described previously. Styles are generally blunt and lobed but there is considerable variation in style morphology. Some species of the *Eudesmia* have anthers in distinct bundles. Some species also have a distinct calyx in the form of four sepals which persist in the fruit.

Monocalyptus

The *Monocalyptus* is characterised by having only a single operculum and virtually all species have ovules arranged in two vertical rows on the placenta. Some *Eudesmia* have two rows of ovules but these are not contiguous. The unit inflorescence is universal in this group. The subgenus is partitioned into two sections *Hesperia* and *Renantheria*. The section *Renantheria* includes all species from Eastern Australia and 4 from Western Australia (*E. brevistylis*, *E. marginata*, *E. staeri* and *E. jacksonii*) and have kidney shaped anthers which dehisce by oblique, fused (confluent) slits. While having similar shape, the anthers of the *Hesperia* dehisce with non- or scarcely confluent slits.

Symphyomyrtus

All *Symphyomyrtus* species have two opercula in contrast to the single operculum of the *Monocalyptus*. In most species, the outer operculum is shed early in development leaving an obvious scar. However, a number of species retain both opercula until flowering. The two opercula may be free, fully-fused or fragmented. Most species have simple axillary inflorescences, although compound inflorescences do occur. The Section *Bisectaria* comprising approximately 100 species is distinguished by having deeply bisected cotyledons while the remainder of species in the subgenus have bilobed or reniform cotyledons. One section, *Adnataria* has adnate anthers compared with the versatile anthers of most of the other sections. *E. microcorys* is treated separately from all of the other members of this genus by (Ladiges and Humphries 1983) as it lacks a sepaline operculum. Both *E. guilfoylei* and *E. microcorys* have incompletely fused petals and they have been shown in analysis of chloroplast DNA to diverge from the *Symphyomyrtus* at a high level (Sale *et al.* 1993).

3. Reproductive Biology

The reproductive process in flowering plants, commences with the initiation of flower buds and continues through their maturation to flowering and the development of fruit and mature seed. The biology of reproduction encompasses the attainment of reproductive competency, floral initiation, the sequence of floral development and factors affecting the success of reproduction and fecundity. A knowledge of these factors is essential for optimising breeding processes. The main flowering traits the breeder is interested in manipulating are the time to first flowering and the abundance of flowering and subsequent seed yield. For many traits and species, the time to first flowering is one of the main obstacles limiting the generation interval and thus the rate of genetic gain per unit time. In addition where seed orchards are being used, it is important to maximise seed yield and quality and minimise establishment, maintenance and collection costs. Seed yield and is maximised through optimising flower bud initiation, retention of the bud crop through flowering and capsule maturation, pollination and seed development .

Table 3.1 Key floral characteristics quantified by Ellis (1991) and Moncur and Boland (1989).

1. Maximum number buds initiated
2. Flowers per umbel at anthesis
3. Open pollinated fruit per umbel
4. No. locules per flower
5. No. ovules per flower
6. No. anthers per flower
7. No. staminodes per flower
8. Length of stamen
9. Diameter of pollen grains
10. No. of pollen grains per flower
11. No. pollen grains/anther
12. Pollen/ovule ratio
13. Mature style length
14. Nectar composition

Age to first flowering

The age at which eucalypts first reach reproductive maturity varies markedly both within and between species and is a major factor limiting the generation interval in breeding programs. In South Africa, the first flowering of *E. grandis* occurs at 2 to 3 years of age, whereas *E. nitens* for example would rarely produce flowers before 10 years (van Wyk 1981b). In Tasmania, *E. nitens* was observed to first flower at four years of age and at six years two thirds of the *E. nitens* families were flowering on one site (Tibbits 1991). The age

of first flowering differs between provenances of *E. globulus*. Flower buds have been observed on plantings of the mallee provenance of this species from Wilson's Promontory after less than one year in the field. However, normal provenances of tree form would not commence flowering until the third year at the earliest and many families and provenances may not flower for at least 5-6 years. On one site Tibbits (1991) observed that 16% of *E. globulus* families had not flowered by 10 years.

While the onset of reproduction is often associated with the transition from juvenile to adult foliage, this is not always the case and there is some evidence to suggest that reproductive and vegetative phase change are independent (Wiltshire *et al.* 1991). In addition, while there appears to be a relationship between plant size and, the age and abundance of first flowering (Cauvin 1983b; Fig. 3.1), there appear to be direct genetic effects controlling flowering precocity *per se* which are independent of plant size. The effect that flowering precocity and fecundity have on later growth of eucalypts is unknown, although negative effects have been reported in other genera (Sedgley and Griffin 1989). The mechanism controlling floral induction and subsequent development are poorly understood in eucalypts, although cold or drought stress may be implicated (Moncur 1992; Moncur and Hasan 1994; Hasan and Reid 1994). Eucalypts are relatively insensitive to day length (Paton 1978), although photoperiod induction of precocious flowering has been reported in one species (Bolotin 1975).

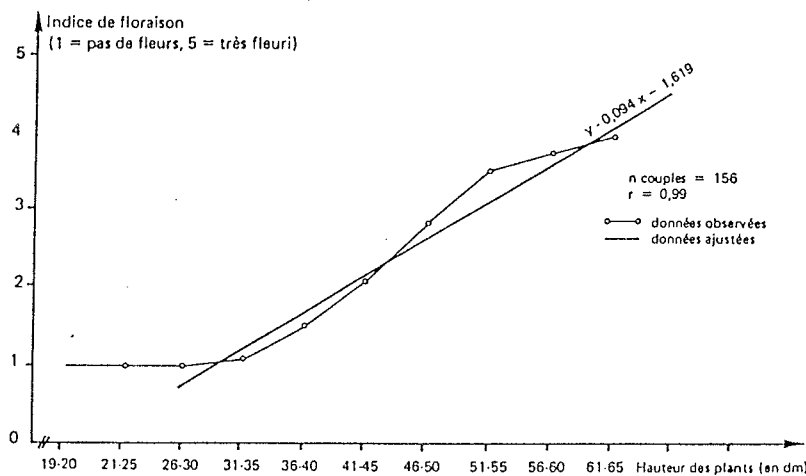


Fig. 3.1 The relationship between flower bud abundance at two years of age and the size of ramets within a single clone of *E. gunnii* (Cauvin 1983b).

Bud initiation and development

The seasonal timing of flower bud initiation varies markedly between species, but in many occurs immediately after the completion of flowering (e.g. *E. globulus*). The time interval between flower bud initiation and flowering ranges from one (e.g. *E. globulus*, *E. nitens*; Fig. 3.2) to two years (e.g. *E. regnans*, Ashton 1975). Even within a single species, populations may differ in the time taken for bud development. In *E. gunnii* for example, buds are retained on trees for two growing seasons in higher altitude populations whereas low altitude populations complete bud development in a single growing season (Potts and Reid 1985).

There are few detailed studies of flower bud development in eucalypts. In *E. melliodora*, buds initiated in late summer (Moncur and Boland 1989), undergo meiosis during the winter months (May-August) after

shedding of the floral bracts, with meiosis occurring first in the anther (Davis 1968). No further changes occur in the male gametophyte during embryo sac development and the first pollen grain mitosis and the development of the generative cell take place after the differentiation of the embryo sac (Davis 1968). The onset of meiosis in *E. stellulata* is associated with a visible enlargement of the buds and a rupturing of the two fused floral bracts which enclose the inflorescence (Davis 1969). Male and female meiosis were virtually synchronous in *E. stellulata*.

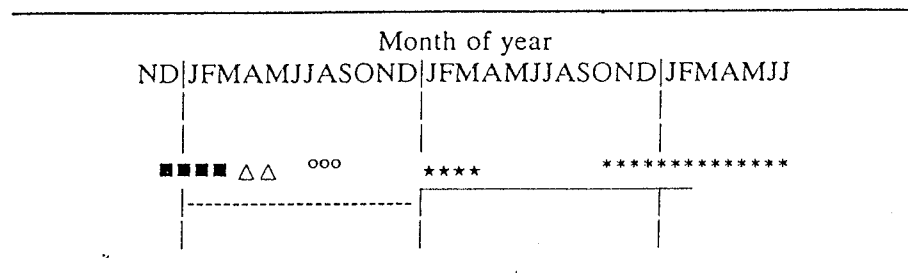


Fig. 3.2 Development of flower and seed crops on *E. nitens* in Tasmania.

The period of initiation of inflorescences (enclosed in bracts; solid squares), bract shed (Δ), flower buds(--), outer opercula shed (oooo), open flowers following inner opercula shed (stars), unripe seed (---) and ripe seed (***) are shown (Tibbits 1989).

Flowering time

The time of onset of flowering also differs markedly between species, and differences in flowering time are a major factor maintaining the integrity of species (Pryor 1976). The peak flowering season may be relatively regular and occur over a relatively defined period of several months, but some species can flower intermittently for up to six months of a year. Flowering within a single stand may occur over a relatively defined period, but there may be considerable variation between stands of a single species resulting in a relatively large spread in flowering time on a geographical basis. In southern Tasmania, natural stands of *E. globulus* commence flowering in late August, peak in October and have generally completed flowering by early January (Fig. 3.3). In contrast, the introduced species *E. nitens* does not usually commence flowering until late December and peak flowering occurs in late January to early February (Tibbits 1989), at a time when virtually all nearby *E. globulus* has finished flowering (Volker 1988).

Within stands of *E. regnans* there is a correlation between the flowering time of neighbours which would favour nearest-neighbour matings (Griffin 1980). Individual *E. regnans* trees flower for 25 to 49 days and there is a range in the time of operculum shed of 4-8 days within umbels, 9-21 days within branches and 9-24

days between branches of single trees (Griffin 1980). The time of flowering within single trees of *E. urnigera* may be spread over 100 days and even within single umbels there is considerable asynchrony (up to 60 days) (Savva *et al.* 1988). Most trees of *E. nitens* complete flowering within three to four weeks and there was generally a consistent ranking of the time of peak flowering between years (Tibbitts 1989).

Variation between provenances may have both an environmental and/or a genetic basis. The environment may influence flowering time. The heat sum experienced after bud initiation may alter the rate of bud development. For example, there is a clear difference in the flowering season for *E. grandis* planted at different altitudes in South Africa (van Wyk 1981b). At high altitudes (1200 m; 1700 mm rainfall), the main flowering season is from April to June whereas at low altitudes (760m; 930 mm rainfall), the corresponding season is from February to March. There is also a trend in natural stands for flowering to be delayed at higher altitudes and there is often waves of flowering along altitudinal gradients (Ashton 1975; Savva *et al.* 1988; van Loon 1966). However, genetic based differences in the flowering time of provenances and families of *E. globulus* have been observed (Volker 1988; Gore and Potts 1995). Provenances from the east coast of Tasmania flowered first whereas those from King Island and the west coast of Tasmania did not commence flowering until one to two months later. The consistent ranking of trees and families within provenances for flowering time across two years suggested that there was a strong genetic basis to variation in flowering time.

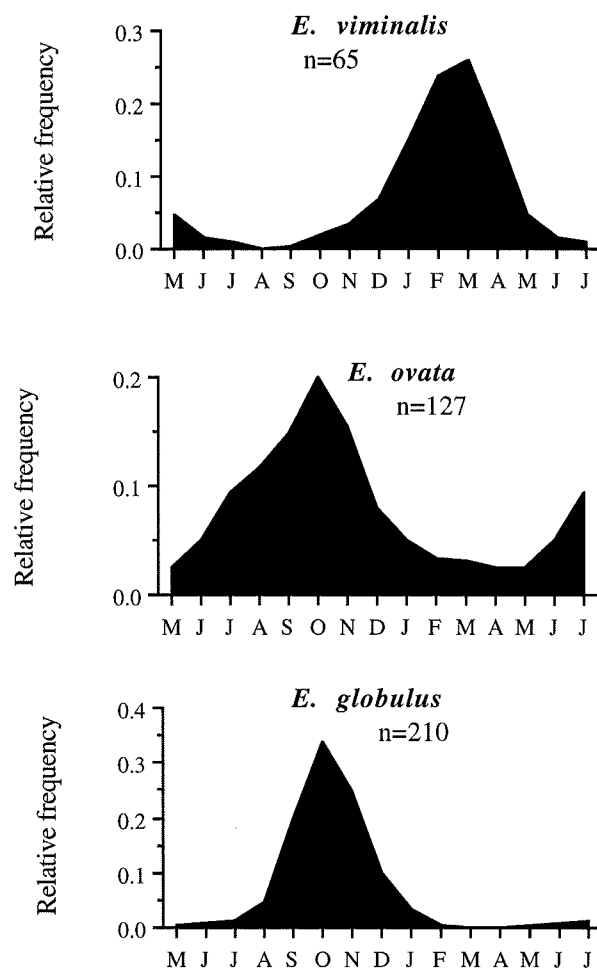


Fig. 3.3 Generalised flowering time curves of three species *E. viminalis*, *E. ovata* and *E. globulus* which are constituents of the lowland dry sclerophyll forests of Tasmania.

Post-anthesis development

Fourteen key stages in the post-anthesis development of the eucalypt flower have been identified by (Moncur and Boland 1989), the duration of which may vary markedly between species (Table 3.2). There is some evidence to suggest that the duration of the phase of floral development is also influenced by environment/climatic factors with low temperatures slowing the rate of floral development (Davis 1969). This is certainly the case for pollen germination and pollen tube growth (Boden 1958).

Table 3.2 The duration of post-anthesis phases of floral development from opercula to staminal abscission (following (Moncur and Boland 1989)). The number of days from the splitting of the operculum (anthesis) is shown. ¹ (Moncur and Boland 1989), ² Polunina cited in (Davis 1968) ³ (Griffin and Hand 1979), ⁴ (Savva *et al.* 1988) (^{3,4} start from operculum shed).

Floral phase	Number of days from opercula lift					
	<i>E. melliodora</i>	<i>E. macarthuri</i>	<i>E. cinerea</i>	<i>E. occidentalis</i>	<i>E. regnans</i>	<i>E. urnigera</i>
Source	1 (mean)	2	2	2	3	4
Operculum shed	1.5	-	-	-	0	0
Staminodes loose	1.9	-	-	-	-	-
Staminodes unfold	2.2	-	-	-	-	-
Stamen unfold	2.9	2	2-3	2-3	1	-
Release of pollen	-	2	2-6	2-12	0	0
Anthers empty	-	4-8	7-11	9-11	5	16
Start wet stigma	2.9	2-5	2-8	2-7	12	13
Start nectar	4.7	-	-	-	4	8
End nectar	8.7	-	-	-	16+	24
End wet stigma	10.0	6-10	12-13	8-12	18+	27
Stamens wither	15.1	8-10	12-14	11-19	-	23
Some stamens abscise	16.6	-	-	-	-	-
All stamens abscise	18.7	-	-	-	-	-
Loss of style	12.9	13-15	30-40	18-23	26+	-
Appreciable enlargement of ovary	-	20-30	40-60	30-45	-	-

Pollination

Pollen shed

The first process in the dehiscence of the operculum is the development of dead tissue marking the top of the hypanthium and the base of the operculum. The expanding stamens force the operculum (or opercula) from the hypanthium and then expand outward (Fig. 2.4). The anthers then dehisce through longitudinal slits or pores, releasing the pollen grains. In *E. melliodora*, the generative nucleus has entered the early prophase of second pollen grain mitosis at the time of anthesis (Davis 1968). In *E. stellulata*, the pollen undergoes mitosis just prior to anthesis and the pollen is liberated in the two-celled condition (Davis 1969). At this stage, the components of the egg apparatus undergo a three-fold increase in size just before anthesis (Davis 1969) but the stigma is not receptive.

In some eucalypt species the pollen adheres to the style in a sticky mass and is deposited on pollinators when foraging for nectar (Boland and Sedgley 1986; Ellis *et al.* 1991). The sticky exudate which clumps pollen is produced by the anther gland (Ellis *et al.* 1991). In species with non-clumping pollen, (e.g. *E. globulus* and *E. nitens*) pollen is transferred directly from the anthers by brushing. There are numerous pollen grains produced per flower (64,000-236,000; Moncur and Boland 1989), and the pollen grain to ovule ratio is high, in the order of 4,000-20,000 to 1 (Moncur and Boland 1989; Ellis 1991). After flowering, the stamens wither and fall and in some species, the staminal ring may also be shed whole or in fragments. There is some

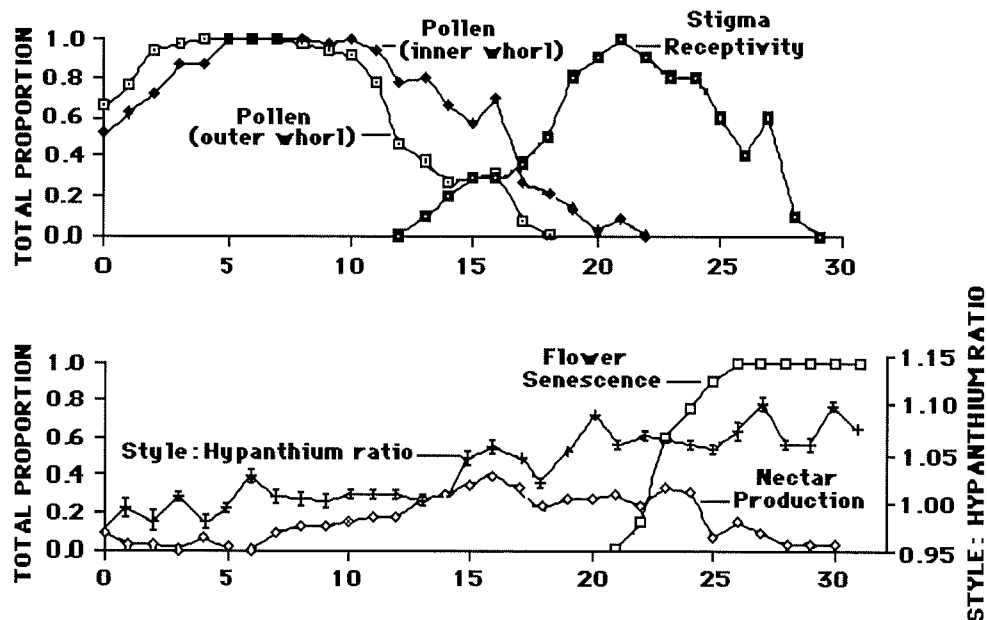


Fig. 3.4 Post-anthesis floral development in *E. urnigera*. The proportion of flowers with (top) free pollen on the inner and outer whorls of stamens, a receptive stigma, (bottom) senescing anthers and the average proportion of the hypanthium filled with nectar and the length of the style relative to the height of the hypanthium (>1 style extending above hypanthium rim) (from Savva, *et al.* 1988).

evidence to suggest the senescence of the stamens is triggered by pollination and/or fertilisation of the flower, and in cases where the stigma or style has been damaged, delayed senescence of the stamens has been observed (Savva *et al.* 1988).

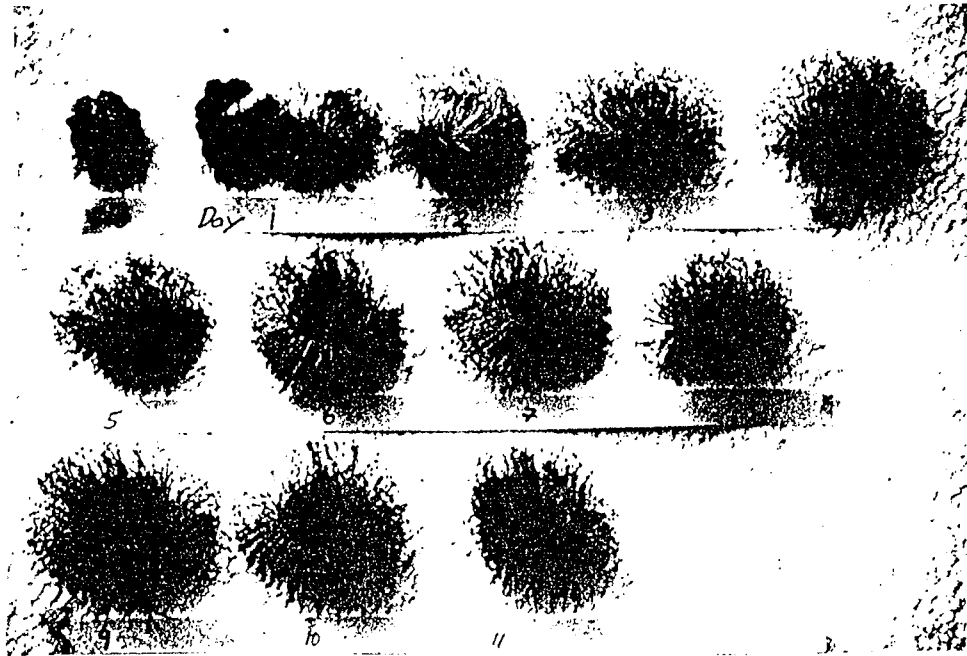


Fig. 3.5 Post-anthesis floral development of *E. globulus* (photo P. Gore)

Pollen physiology

Eucalypt pollen is triangular and tri-colpate (Fig. 3.6), and differences between species in size (e.g. Gore *et al.* 1990) and some microstructure features between species have been reported (e.g. Churchill 1968). The pollen is robust (Pryor 1976) and resistant to the osmotic stress imposed by immersion in water, and is extremely heat tolerant in the dessicated state (Heslop-Harrison and Heslop-Harrison 1985). The pollen will germinate over a range of temperatures, with an optimum between 20-30°C and a rapid decline with temperatures above 35°C (Boden 1958). A small percentage of *E. gunnii* pollen will germinate at 5°C although the optima was around 30°C (Marien 1988). The fact that pollen will germinate over a wide range of temperatures enables pollen germination and pollen tube growth to proceed regardless of weather conditions. Boden (1958) notes that at low temperatures the rate of germination and pollen tube growth is slow and that it is doubtful whether fertilisation would result if temperatures were consistently below 4°C. However, pollen tubes which have commenced growth will respond to temperature increase and in cold regions, there is probably diurnal fluctuations in tube length extension. The extent to which pollen tubes will

be affected by freezing temperatures is unclear, although Marien (1988) has reported damage to hydrated *E. gunnii* pollen at -4°C .

Fig. 3.6 Pollen grain of *Eucalyptus melliodora* (Moncur and Boland 1989).



Pollen germination and pollen tube growth

After the flower opens (losses the operculum), the pollen collects on the stigma. Pollen grains adhere and germinate in the sticky mucilaginous secretion on the stigmatic surface. There is usually little stigmatic exudate at anthesis, but between 5 and 10 days after anthesis, the stigma is usually visibly moist and sticky (Griffin and Hand 1979; Savva *et al.* 1988; Sedgley and Smith 1989). The stigmatic exudate contains both carbohydrates and lipids (Anderson 1984; Sedgley and Smith 1989) and it is in this medium that pollen germination occurs.

Fertile pollen grains produce tubes which descend via the style to the ovaries where they effect fertilisation by the fusion of the male nucleus with the female nucleus contained in an ovule. The pollen tubes grow intercellularly through the transmitting tissue and become concentrated into the relatively narrow tract at the base of the style (Gore *et al.* 1990; Sedgley *et al.* 1989; Sedgley and Smith 1989). The pollen tubes do not grow in the stylar canal which may persist for up to two thirds the length of the style and is hollow in the *Monocalyptus* species. In the case of *E. globulus*, the stylar canal is solid and pollen tubes are confined to the transmitting tissue surrounding the stylar canal for the first 1 to 6 mm (Fig. 3.7a). At approximately 6mm the transmitting tissue narrows inwards and the tubes converge into the center of the style (Fig. 3.7b). This arrangement continues to the base of the style. At approximately 1 mm below the base of the style the tubes radiate outwards and grow down the ovary in the tissue surrounding the locules (Fig. 3.7c). In *E. woodwardii*, the pollen tubes have been shown to grow through the septa between the locules, being unable to enter the locule through any other route due to the thickened cuticle lining the inner surface (Sedgley and Smith 1989) (Fig. 3.8). From the septa, the tubes enter the placenta and then grow outward to penetrate the ovules.

Fig. 3.7 The growth of *E. globulus* pollen tubes through the *E. globulus* style. (Gore, et al. 1990)

(a) transverse section 2mm down the style of *E. globulus*, showing the lobed canal (L), pollen tubes (P), transmitting tissue (T), vascular bundles (V) and oil bodies (O). (b) transverse section through the base of the style showing pollen tubes, sclerenchyma (S) and oil bodies (O). (c) transverse section 1.5 mm below the base of the style showing pollen tubes and locules (LO).

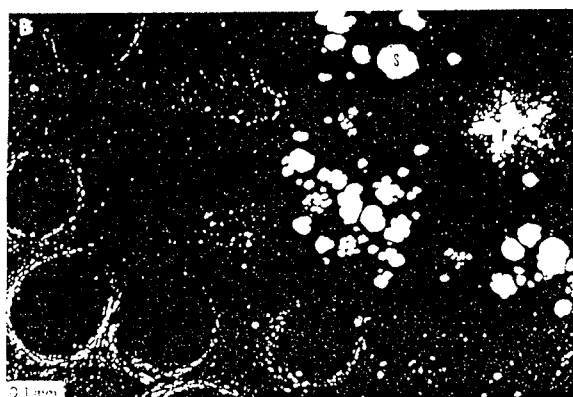
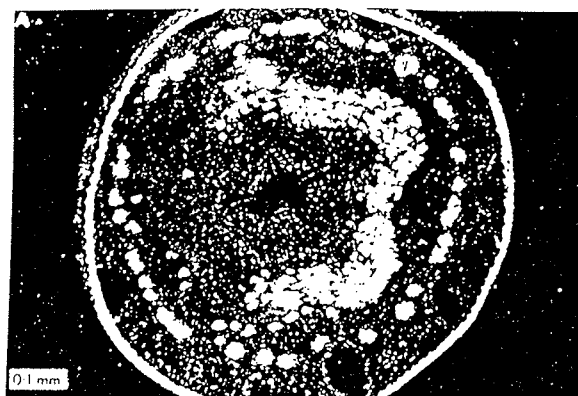
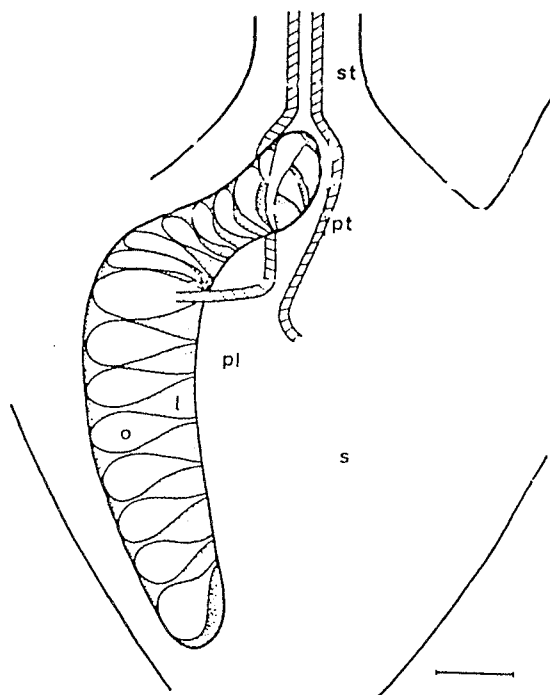


Fig. 3.10 A diagram of the pollinated pistil of *E. woodwardii* (*Symphyomyrtus*) showing the route of pollen-tube (pt) growth in the lower style(st), along the surface of the locules (l), through the septa separating the locules (s), into the placenta (pl) and towards the ovule (o) (from Sedgley and Smith 1989).



Stigma receptivity

The stigma becomes receptive when most of the pollen has been shed from the anthers of the same flower (Table 3.2 ; Fig. 3.4). The opportunity for self-pollination within a flower is thus minimised and the flowers are termed protandrous. However, with considerable variation in flowering time between flowers of the same tree and even the same inflorescence, there is ample opportunity for self pollination from other flowers on the same tree, termed geitonogamous pollination (Griffin 1980). Receptivity is associated with the stigma becoming sticky and sometimes flattening and the release of nectar. In some species, the style may also elongate markedly just prior to receptivity, reaching maximum length at receptivity (e.g. *E. regnans*, Griffin and Hand 1979).

Fertilisation and ovule development

In angiosperms, the pollen tube enters the ovary via the micropyle and both male gametes are involved in fertilisation, one fusing with the egg to form the diploid zygote, the other fusing with one or more of the polar nuclei to form the endosperm. However eucalypt seed does not contain obvious endosperm and thus the endosperm tissue must be absorbed relatively early in ontogeny.

In *E. regnans* (subgenus *Monocalyptus*), Sedgley *et al.* (1989) observed sperm nuclei in the embryo sac 1 week after pollination and the nucellus and embryo sac of unfertilised ovules had collapsed by 8 weeks. Embryos with up to 16 cells were present by 16 weeks after pollination. Boland *et al.* (1980) and Boland *et al.* (1985) consider that ovules towards the centre of the placenta do not get fertilised. This may be correct for species with greater than two rows of ovules, however, in the case of the *Monocalyptus* species, *E. regnans*,

there was no relationship between the position of the ovule on the placenta and either penetration of the ovules or the occurrence of fertilised ovules (Sedgley *et al.* 1989).

Capsule maturation, dehiscence and seed shed

After fertilisation, the flower enlarges, dries and becomes a woody fruit. In many species the style is shed, in others it remains as a short point on the roof of the ovary chambers. In most species, the roof of the ovary dehisces by splitting radially forming valves which retract to allow the shedding of the seed (Boland *et al.* 1980). In natural forests of *E. regnans*, seed is shed throughout the year but there is a peak in late summer (Cremer 1965a). The length of time that mature seed is retained on tree varies markedly with species. Many temperate species may retain two to three years capsule crops in the canopy whereas species of the subgenus *Blakella* shed seed rapidly sometimes within days of flowering (Boland *et al.* 1985). Seed shed is accelerated by drought or fire and retarded by wet conditions (Boland *et al.* 1980).

The number of viable seed obtained per capsule under open pollination varies between species but in *E. nitens* (Tibbits 1989) *E. regnans* (Eldridge and Griffin 1983) and *E. globulus* ranges from 2-4 in capsules. However, following controlled outcrossing seed set per capsule is increased in virtually all species examined which suggests that the amount of outcrossed pollen transferred to the stigma may be a factor limiting seed set in natural populations.

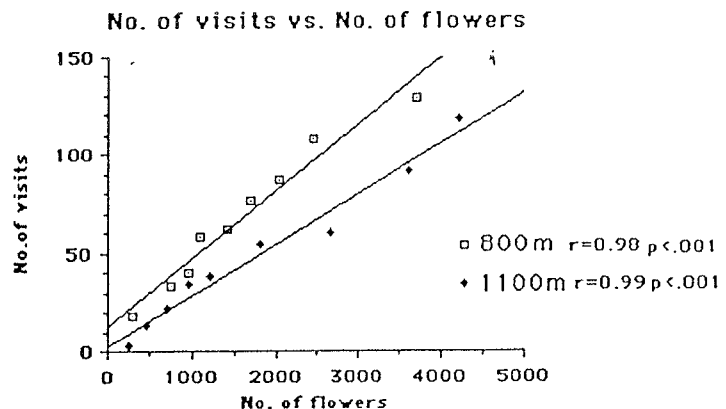
4. Breeding system

Pollen vectors

In contrast to seed dispersal, the breeding system of *Eucalyptus* appears to be well adapted for pollen-mediated gene flow (Barber 1965; Pryor 1976). Pollination is effected by a wide variety of active, often generalist bird, insect and marsupial species (Ashton 1975; Barber 1965; Griffin 1982; Hopper and Moran 1981; Ireland and Griffin 1984; Savva *et al.* 1988). Wind pollination has been reported in *E. teriticornis* (Pryor 1976), but this requires verification.

The attractiveness of a tree will depend upon the nectar reward for pollinators. In *Eucalyptus*, the number of flowers produced per tree differs, ranging from 100 in *E. stoatei* (Hopper and Moran 1981) to more than 1.5 million in the mass flowering species *E. regnans* (Griffin 1982). Within a species where flower bud size is relatively constant, the frequency of pollinator visits per tree is directly dependent on the number of flowers per tree (Fig. 4.1). Flower bud size as measured by the width of the hypanthium is believed to be a good indication of the magnitude of the nectar reward/flower for pollinators and filament colour is the most obvious secondary attractant (Griffin 1982). There is a strong association between coloured flowers and large flower size in the genus, both of which are floral syndromes favouring bird pollination. Smaller flowered eucalypt species, which may provide net calorie rewards to insect visitors only, tend to have white flowers and flower during late spring/autumn when insects are relatively more active. The larger flowered species which attract the larger, more active bird visitors, have a higher proportion of species with brightly coloured flowers and tend to have less clearly defined flowering peaks, but many tend to be at peak flowering during the colder time of the year (Griffin 1982). The anthers are a basic attractant as emasculated flowers which have not been isolated tend to yield negligible quantities of seed (van Wyk 1981b).

Fig. 4.1 The relationship between the number of bird pollinator visits and the number of flowers per tree of *E. urnigera* at two sites (Savva *et al.* 1988).



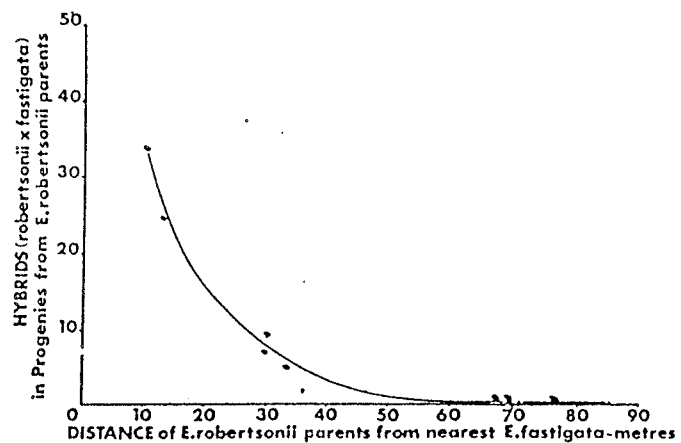
Pollen dispersal

While there is very little quantitative information on pollen-mediated dispersal in *Eucalyptus* as with seed, most pollen is probably deposited in relatively close proximity to its source (Potts 1990; Savva *et al.* 1988). Birds tend to follow a close to nearest-neighbour foraging pattern (e.g. Hopper and Moran 1981; Savva *et al.* 1988), although pollen may be carried over for several trees (Savva *et al.* 1988). Nevertheless, as noted by

(Barber 1965), gene flow in *Eucalyptus* is probably more a function of pollen than seed, and it is particularly in the frequency of rarer long distance dispersal events, namely the tail of the dispersal curve, that pollen probably surpasses seed.

Over five percent of all inter-tree foraging movements by birds involve long-distance (out-of-sight) escape or exploratory flights (Hopper and Moran 1981; Savva *et al.* 1988). It is difficult to assess exactly how far the tail of the pollen dispersal curve extends. Progeny analysis, using interspecific hybrids as genetic markers is one of the most practical ways of assessing pollen dispersal (Fig. 4.2; Potts and Reid 1988). Rare hybrids with lowland species are regularly found in eucalypt populations on mountain summits in cases nearly 5 kilometers from the nearest source of pollen (Potts 1990). The tail of the pollen dispersal curve is probably extremely long, particularly where bird pollination is involved, and information from the distance and frequency of interspecific hybrids is likely to underestimate the frequency of long distance pollen dispersal within species.

Fig. 4.2 The distance of pollen travel between pairs of trees as revealed by hybrid progeny in seed lots of *E. robertsonii* contaminated by pollen of *E. fastigata* (Pryor 1976)



Seed Dispersal

Seed dispersal in most eucalypt species is mainly by wind and gravity (Cremer 1965b, 1977), although there is a recent study which suggests that one species may be bee dispersed (Wallace 1991). With the possible exception of the subgenus *Corymbia* neither the seed nor fruits exhibit special adaptations to wind and gravity dispersal (Cremer 1977). Seed of most of the *Corymbia* species from the section *Rufaria* have rudimentary wings that are derived from the seed coat, but there is no published evidence that they are a functional adaptation for dispersal (Boland *et al.* 1980). Studies of seed fall from commercial species indicate that virtually all seed is deposited within a radius of twice the tree or canopy height (Cremer 1965b, 1977; Potts 1990). The distance of seed fall is essentially proportional to canopy height, seed weight (i.e. terminal velocity) and wind speed (Cremer 1977). The movement of seed once deposited on the ground is probably fairly limited in most species, although extensive seed harvesting by ants no doubt results in some effective dispersal.

Estimates of the standard deviation of seed dispersal distances (σ_{seed}) for the tall forest species, *E. regnans*, range from only 30 to 48 m (Table 4.1). The distance of seed fall decreases with canopy density and, under normal circumstances, it is unlikely that even these distances would be achieved with a closed canopy. Furthermore, most of these measurements were taken downwind of a seed source whereas recent studies indicate a marked, asymmetry in seed dispersal with a nearly 15 fold increase in the number of seed dispersed downwind of a source. Nevertheless few species achieve the height of *E. regnans*, and seed dispersal in the majority of eucalypt species is likely to be considerably less. Limited seed dispersal may result in the progeny of a female tree becoming established in the forest in close proximity to the parent resulting in the formation of family group structures (sibs or half-sibs) in native stands of some species (Eldridge *et al.* 1993, p. 195; Hardner *et al.* 1995).

Table 4.1 Estimates of seed dispersal (standard deviation) in eucalypts (Potts 1990).

Species	Seed Dispersal σ_{seed} (m)	Canopy Height (m)	Comments
<i>E. risdonii</i>	14.6	2.5	(from boundary)
<i>E. regnans</i>	17.5	55.0	(SE of 2 trees)
	41.2	76.2	(SE of 2 trees)
	30.3	61.0	(SSE of dense forest edge)
	31.6	61.0	(SSE of dense forest edge)
	45.1	61.0	(SE of sparse forest edge)
	48.4	61.0	(SE of isolated tree, adjusted canopy height)

Rare long distance dispersal events may occur by several means. Under abnormal circumstances seed or capsules may be dispersed by birds, floods, storms, or in fire updrafts. Most eucalypt species are dependent on fire for natural regeneration and seed dispersal during non-fire periods may be markedly less than that during wildfire. Small branches with capsules attached may be carried in fierce convectional updrafts during crown fires and deposited some distance from parent trees. This is more likely to be significant in the wetter forests, although in the drier, more open forests high winds frequently blow capsules attached to small branches and twigs considerable distance along the forest floor. This is possibly one of the main modes of longer distant seed dispersal in the drier forests. While strong beaked birds may break open woody capsules to eat seed, their significance as a mode of dispersal has not as yet been demonstrated. Water transport may also be significant, particularly for species growing on river margins or floodplains (e.g. *E. camaldulensis*).

Karyotype

Virtually all species within the subfamily Leptospermoideae, to which *Eucalyptus* belongs, have a haploid chromosome number of $n=11$ with the remainder of species (approx. 8%) with $n=22$ (Barlow 1981). All *Eucalyptus* species have extremely small chromosomes and those examined to date have $n=11$ (Ruggeri 1960; Rye 1980; Haque 1984). While there are reports of artificial induction of allotetraploids (Kapoor and Sharma 1984), there are no reports of natural polyploidy or variation in chromosome number. No abnormal patterns were observed during meiosis in an artificial interspecific F_1 hybrid between *E. cinerea* and *E. maculosa* suggesting similarity in chromosome structure between the two species (Mergen *et al.* 1966).

Levels of outcrossing

Eucalypts are generally preferentially outcrossing (Pryor 1976; Griffin *et al* 1987), with high outcrossing rates (e.g. 0.69-0.84 Moran and Bell 1983; Prober *et al.* 1990) maintained by varying degrees of self-fertility (Pryor 1976), aided by protandry (Griffin and Hand 1979; Fig. 3.2) and reinforced by selection against the products of self-fertilization in later stages of the life cycle (Potts *et al.* 1987; Hardner and Potts 1995).

Self fertility

Most species exhibit a marked reduction in seed yield following self-pollination compared to outcrossing, although within species there is considerable variation in the level of self-fertility (Pryor 1957; Pryor 1976; Table 4.2). In most of the species examined to date, the majority of individuals are partially self-fertile, but individuals range from fully self-incompatible to fully self-fertile. Post-mating barriers to self-fertilization are thus rarely complete, and (Eldridge 1976) notes that "persistent attempts at artificial self-pollination have been successful to some degree on almost every tree tested". Griffin *et al* (1987) found that with only one possible exception, all the *E. regnans* trees investigated were self-fertile to some degree as were the trees of *E. grandis* tested by Hodgson (1976c). Pryor (1957) found that of some dozens of individuals examined of nine species only one tree of *E. bicostata* was found to be fully self-incompatible. Early reports of self-sterile species (Krung and Alves 1949) have not been substantiated (e.g. *E. grandis* - Hodgson 1976a and 1976c) and clearly adequate controls and several independent replications of a cross are required before a plant can be reliably classified as fully self-incompatible.

Table 4.2 The level of self-incompatibility in some eucalypt species (modified from Potts and Savva 1988).

The overall level of self-incompatibility (SI) is measured as the percentage reduction in seed yield (per flower crossed or capsule harvested) following controlled self-pollination compared to outcrossing and is based on grand means for each pollination type. The classification of individuals was based on either the assignment reported or the calculated level of self-incompatibility (fully self-incompatible SI > 95%, partially self-incompatible 5% < SI ≤ 90% or fully self-compatible SI < 10%). (¹further testing required)

Species	level of SI (%)		Percentage of trees			N	Source
	seed/flower	seed/capsule	fully SI	partially SI	fully self-fertile		
<i>E. regnans</i>	48	47	7	64	29	14	Eldridge and Griffin 1983
	69	41	0	80	20	5	Griffin <i>et al</i> 1987
<i>E. grandis</i>	-	79	0	100	0	11	Hodgson 1976b
<i>E. ovata</i>	84	73	0	86	14	7	Potts unpubl. data
<i>E. nitens</i>	77	72	¹ 14	86	0	14	(Tibbits 1989)
<i>E. globulus</i>	74	75	8	77	15	13	(Potts 1988)
<i>E. gunnii</i>	81	73	20	60	20	10	(Potts and Savva 1988)
<i>E. morrisbyi</i>	100	-	100	0	0	4	diallel " "
			84	11	5	19	self c.f. O.P.
<i>E. pulverulenta</i>	-	-	30-40	40-50	20	10	Pryor 1976
<i>E. woodwardii</i>	-	93	-	-	-	2	(Sedgley 1989)

In *E. globulus*, there was no significant difference in seed set between assisted (emasculated and self pollen manually applied) and non-assisted self-pollinations (flowers simply bagged without emasculation) (Fig. 4.3). However, there was a significant reduction in seed set following self-pollination compared to intraspecific outcross, polycross and open-pollinations (Fig. 4.3). The number of seeds set per flower or per capsule following assisted self-pollination was 74% ($P < 0.05$) and 75% ($P < 0.001$) less than following outcrossing respectively. There was considerable variation amongst individuals in the level of self-incompatibility with individuals ranging from fully self-fertile to fully self-incompatible (Table 4.2). However, of the 13 trees on which both self and outcross pollinations were performed, the majority (77%) were partially self-incompatible exhibiting a 73-97% reduction in seed set following selfing. Only one tree (8%) was fully self incompatible and only two trees (15%) were fully self-fertile. Similar results have been reported for *E. nitens* (Tibbits 1989).

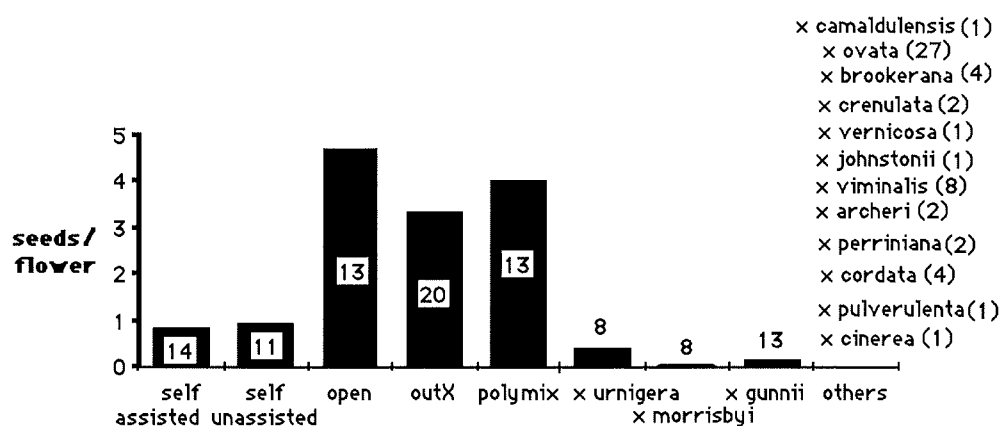


Fig. 4.3 The mean number of viable seed obtained per flower crossed for intraspecific and interspecific crosses of *E. globulus* (Potts and Savva 1989).

Mechanism of self-incompatibility

The mechanisms controlling preferential outcrossing and by which seed set is reduced following self pollination appears to differ between species. In virtually all cases, self pollen tubes appear to grow successfully to the base of the style. In *E. regnans*, the species studied in most detail to date, preferential outcrossing appears to be mainly controlled by postzygotic mechanisms (Griffin *et al* 1987). No consistent differences in pollen tube growth following self- and cross-pollination was observed in the styles and there was no difference between self- and cross-pollinations in the number of ovules penetrated or the number of fertilised ovules at 16 weeks following pollination (Griffin *et al* 1987). However, there was a marked reduction in seed set following selfing and when pollinated with 1:1 self:outcross pollen mix, 80% of the seed obtained from this species was from outcrossing (Griffin *et al* 1987). The proportion of outcross seed obtained was much higher than expected on the basis of independent crosses. The mechanism favouring preferential outcrossing in this species appears to be post-zygotic and appears to be dependent on embryo genotype and maternal resource allocation (Griffin *et al* 1987). By contrast, in *E. woodwardii*, the major barrier appears to operate at the time of ovule penetration with post-zygotic seed abortion only playing a minor role in the controlling seed number (Sedgley 1989). The genetic basis of variation in self-fertility is unknown.

Male sterility

Male sterility is another significant mechanism by which self-fertilisation is prevented in natural eucalypt populations. While the eucalypt flower is usually bisexual and trees hermaphrodites, there are an increasing number of reports of trees in which the anthers are sterile and all flowers are functionally female. These trees are thus obligate outcrossers. Significant levels of unisexual, male sterile individuals have been reported in *E. pulverulenta* (Peters *et al.* 1990) and *E. leucoxylon* (Ellis 1991). Pryor (1976) also notes occasional individuals of *E. grandis* with infertile pollen and Davis (1969) records that a high proportion of flowers on some trees of *E. stellulata* were male sterile.

Effects of inbreeding

All eucalypt species studied to date have shown marked inbreeding depression following self pollination (S_1 ; $F=0.5$). In *E. nitens*, significantly poorer germination and higher percentage abnormal seedlings and mortality occurred in glasshouse grown progenies from self and open pollinations compared to outcrossed progeny (Tibbits 1988; Table 3.5). Significantly greater mortality of selfed and unrelated outcrossed progenies of *E. gunnii* were detected in the nursery and after 1 years' field growth the height

Table 3.5 Percentage germination, and percentage of abnormal seedlings and mortality in glasshouse grown self, open pollinated and outcrossed progenies of *E. nitens* (Tibbits 1988).

Cross type	-----Percentage-----		
	germination	abnormalities	mortality
Outcross	88a	1.1a	4.4a
Open	79ab	8.8b	5.0a
Self	74b	6.4b	20.0b

of selfed progenies was significantly less than outcrosses with open pollination progenies intermediate (Potts *et al.* 1987). Inbreeding depression for height growth of selfed progeny of *E. grandis* varied from 8-49% and there was a reduction in the performance of open pollinated progenies of 8-13% (Hodgson 1976c). Detailed studies of the effects of inbreeding *E. regnans* have been undertaken by (Eldridge and Griffin 1983; Griffin 1990; Griffin and Cotterill 1988; Hardner and Potts 1995) comparing selfed, open-pollinated and outcrossed progenies. Little difference in growth was detected between the cross types at three months, but by 11 months in the field selfed progenies were significantly shorter. Inbreeding depression for growth traits tends to increase with age and at 45 months inbreeding depression in diameter (11%), height (18%) and conic volume (37%) have been reported (Griffin and Cotterill 1988). Even greater inbreeding depression has been reported in *E. globulus* with inbreeding depression due to selfing resulting in reductions of 24% in diameter, 26% in height and 48% for conic volume at 43 months (Hardner and Potts 1995).

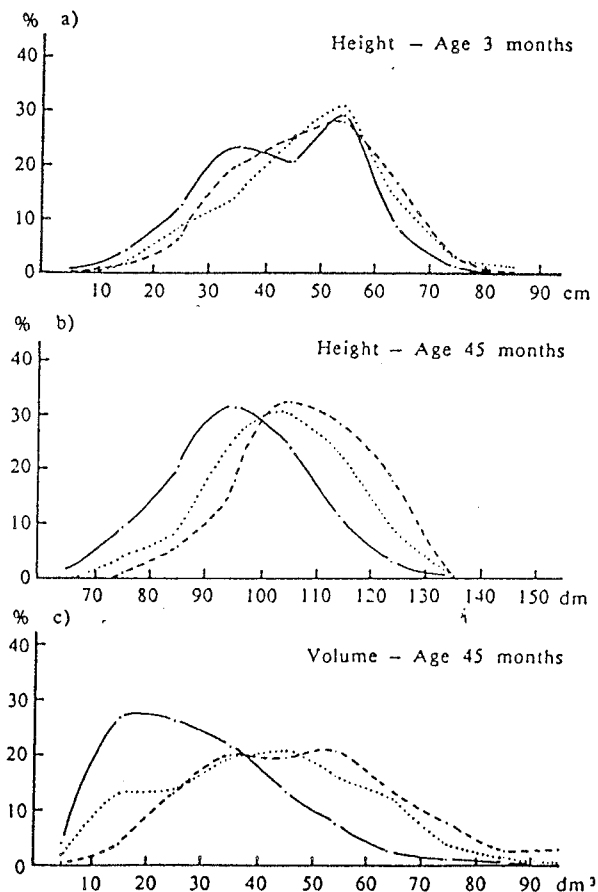
In all cases, significant depression of the performance of open pollinated progenies have been detected compared to outcrossed which is believed to be due to inbreeding (12-23% for volume; Griffin and Cotterill 1988; Hardner and Potts 1993 and 1995). Inbreeding in natural forests can arise if -

- (i) the population size is small
- (ii) there is assortive mating between individuals of like phenotype (e.g. similar flowering time)

(iii) there is a tendency for assortive mating between relatives (e.g. sib matings or in the extreme case selfing) (Griffin 1990).

In the latter case, while eucalypts are preferentially outcrossing, the mechanisms preventing self fertilisation in eucalypts do not appear to be of sufficient strength to eliminate all selfed progeny from open pollinated seed lots prior to dispersal. While in competition with outcrossed pollen, the preferential outcrossing mechanisms favour outcrosses over self pollinations. However, temporally or spatially isolated trees may still successfully reproduce, although a large proportion of the open-pollinated seed lots would be the products of self-fertilisation. In addition, the limited seed dispersal in the genus would suggest that spatially close individuals in a forest are likely to be related to some degree.

Fig. 4.4 Size class distribution of Selfed (——), outcrossed (-----) and open pollinated (.....) trees in a trial of *E. regnans* at 3 and 45 months after planting (Griffin 1989b, Griffin and Cotterill 1988).



The nearest-neighbour foraging patterns of the pollinators would tend to favour matings between spatially and potentially genetically close individuals. This would mean that open pollinated seed lots could contain progenies which are the products of full-sib ($F=0.25$) or more likely half-sib ($F=0.125$) matings in addition to selfs ($F=0.5$). The only indication to date of the magnitude of this neighborhood inbreeding effect is provided by the study of (Moran *et al.* 1989) using *E. regnans*. The outcrossing rate estimated in a first

generation seed orchard in which progenies from 40 open pollinated seedlots were planted in a random mixture was $t = 0.91$ compare with a value of $t = 0.75$ from the native stand. The selfing rate can be assumed constant and the difference between the two estimates must reflect levels of inbreeding associated with neighborhood effects. While the effect of less extreme levels of inbreeding on growth are unlikely to be as extreme as than reported for selfing, significantly poorer performance of full-sib matings of *E. grandis* compared with unrelated matings have been reported (van Wyk 1981a). Poorer performance of related outcrosses compared to unrelated outcrosses have also been reported in *E. gunnii* (Potts *et al.* 1987). The relationship between the level of inbreeding (F) and inbreeding depression has not been quantified in eucalypts, but work with conifers suggests that the relationship is linear and that for every 0.1 increment in F there is on average a 4.5% reduction in early growth. If a linear relationship is assumed in eucalypts then we can expect a 10% decrease in early volume of *E. globulus* with every $F = 0.1$ increase in inbreeding. It thus appears that even without selection, major genetic gains in vigour can be achieved initially in a breeding program simply by removing the inbreeding in a base population.

In natural forests, there would be intense competition between outcrossed and inbred products and the results of progeny trials leave little doubt that intense competition operating over the life cycle would rapidly eliminate the vast majority of inbred products from the mature stands (Hardner and Potts 1995). Most eucalypts depend on wild-fire for regeneration and in the case of *E. regnans*, for example there is a reduction in stand density from over 350,000 stems per hectare after fire to less than 1000 in the first twenty years of growth (Griffin 1990) allowing ample opportunity for the elimination of inbred products at little selective cost. At reproductive maturity the mature forest is thus likely to be dominated by highly outbred individuals. Natural selection would favour the products of more long distant pollinations and thus there is likely to be a large discrepancy between pollen dispersal and actual effective gene flow.

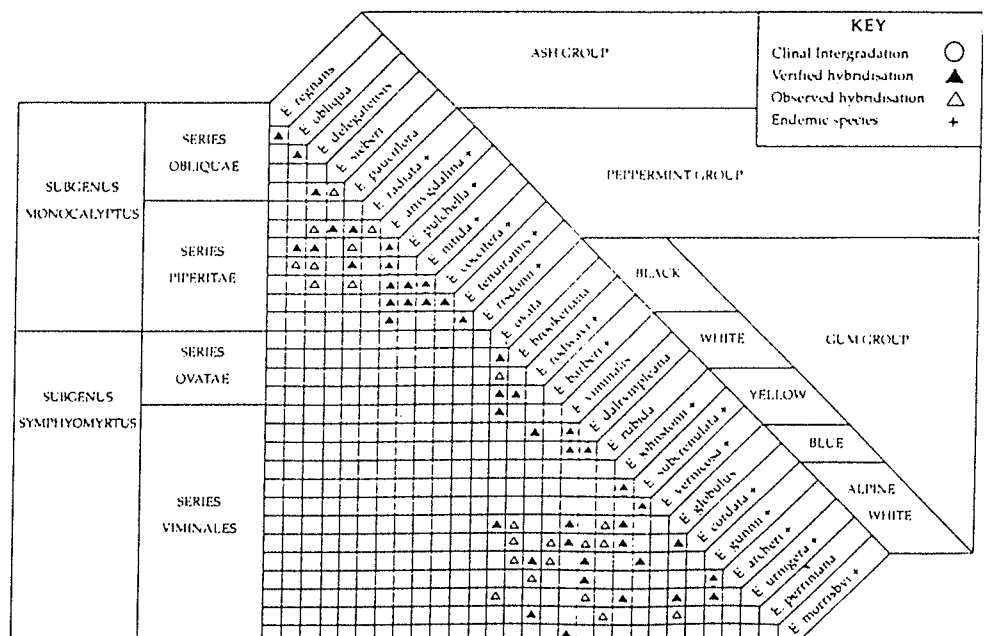
5. Interspecific hybridisation

Natural hybridisation

Interspecific hybridisation often occurs in natural populations of *Eucalyptus*, and is believed to be a significant evolutionary process in the genus (Potts and Jackson 1986; Potts and Reid 1988; Whitham *et al.* 1991). Indeed, many of the taxonomic difficulties in the genus are believed to be due to hybridisation and the relatively weak reproductive barriers between species, at least within sections (Pryor and Johnson 1971; Pryor 1976). However, following a review of the occurrence of hybridisation in the genus, (Griffin *et al.* 1988) suggest that despite the reputation of the genus for hybridisation, natural hybridisation is a fairly restricted phenomenon. Of the 528 species examined, 289 (55%) were recorded as being involved in at least one hybrid combination. However across the genus, natural hybridisation was found to be rather restricted with only 15% of combinations expected on geographic grounds having been recorded.

Natural and artificial hybridisation between species from the different subgenera does not occur (Pryor and Johnson 1971) although within subgenera hybridisation is relatively common (Griffin *et al.* 1988) (Fig. 5.1). However, within subgenera the extent of hybridisation may vary considerably, depending upon the co-occurrence of species, the degree of overlap in flowering and pollinators, ecological differences and post-mating breeding barriers. (Griffin *et al.* 1988) ranked all pairs of species according to their taxonomic difference and the distance between the two species in the nearest part of their natural range and compared these traits to the frequency of reported natural hybrids. A total of 520 hybrid combinations were reported and virtually all species involved grew in close geographic proximity. No natural hybrids were recorded with the *Idiogenes* (monotypic), *Gaubea* (two species) or *Teleocalyptus* (four species) subgenera. However, within the other subgenera, the frequency of natural hybrids generally reflected the hierarchy of taxonomic affinities. The frequency of hybrid combinations thus decreased depending upon whether the parental species were from the different sections within the subgenera, different series within the same section or from the same series. Important exceptions were recorded in the *Monocalyptus* and *Corymbia* where intersectional hybrids were as common as intrasectional hybrids.

Fig. 5.1 Natural hybrid combinations reported amongst the Tasmanian *Eucalyptus* species. (Duncan 1989)



Within the *Symphyomyrtus*, the series *Ovatae* of the section *Maidenaria* and the series *Olignthae*, *Odoratae* and *Pruinosae* of the section *Adnataria* were identified as having a relatively high ratio of interseries to intraseries hybrids. The predominantly eastern sections *Transversaria*, *Exertaria* and *Maidenaria* have equivalent rates of intersectional hybridisation (1.63%) whereas the western sections *Bisectaria* and *Dumaria*, noted for their low hybridisation rates, average only 0.23% and the section *Adnataria* only 0.43%. The latter sections thus appear to be more isolated from other sections within the *Symphyomyrtus*.

Hybrids may occur as sporadic individuals generally found along the boundaries of natural stands, in hybrid swarms where hybridisation has extended beyond the first generation or in zones of introgression. Introgression zones usually arise when two previously geographically isolated taxa come into contact and there is no strong genetic barrier preventing hybridisation and extensive backcrossing. However, after many generations, it is difficult to differentiate such cases from primary clinal intergradation arising from selection from within the gene pool of a single species (Potts and Jackson 1986).

Several criteria have been suggested for the identification of hybrids in natural populations (Table 5.1) and molecular markers in both chloroplast and nuclear DNA when used together will also prove valuable.

Table 5.1 Criteria listed by (Hopper, et al. 1978) as relevant to the identification of natural hybrids in *Eucalyptus*.

1. Intermediate morphology
2. Phenotypic segregation in progeny
3. Occurrence in sympatric parental stands
4. Close resemblance between suspected and manipulated hybrids
5. Occurrence in disturbed or relatively youthful habitats
6. Impaired reproductive capabilities compare to parental individuals
(i.e. F2 breakdown)
7. Occurrence of interspecific pollen exchange by indiscriminant vectors
in sympatric stands

Artificial hybridisation

There is increasing interest in the use of eucalypts hybrids in forestry, stimulated by the success of hybrid breeding programmes in the Congo and Brazil (Griffin, et al. 1988, Martin 1989, Nikles and Griffin 1992). The eucalypt hybrids in operational use around the world have been reviewed by (Martin 1989). In the Congo, the F1 hybrids *E. teriticornis* x *E. grandis* and *E. alba* x *E. urophylla* are superior to the parental species and widely planted. The *E. grandis* x *E. urophylla* hybrid is extensively planted in Brazil.

The terminology and concepts associated with hybrid breeding in forest trees have been addressed by (Nikles and Griffin 1992) and (Sedgley and Griffin 1989). F₁ hybrids are the first generation hybrids derived directly from crossing the two parental species (AxB). In crop plants, hybrids usually involve crosses

between highly inbred, homozygous lines, and the F_1 is usually highly heterozygous. In most forest trees, the parents are already likely to be highly heterozygous which may result in relatively variable F_1 hybrid families, although on simple genetic grounds it should be no more variable than either parent. Various types of advanced generation hybrids can be derived from the F_1 , including (a) F_2 , F_3 , etc. hybrids and (b) backcross hybrids. The F_2 , F_3 ,... hybrids are derived from successive generations (1,2,... respectively) of crossing amongst the hybrids. It is also important to distinguish between later generation hybrids which involve different levels of inbreeding. For example, F_2 hybrids may be derived by selfing F_1 's, crossing amongst full-sibs from the same F_1 family or by crossing unrelated F_1 hybrids (Fig. 5.2). Backcross hybrids are produced by mating the F_1 hybrid to individuals in the F_0 generation belonging to one or other of the parental species. F_1 hybrids are rarely used in fruit tree breeding for example, where three or four generations of backcrossing are required to recover the desired combination of traits (Griffin 1989a). Hybrids of different family structure and degree of relationship can also be produced depending on the mating design which include open pollination, 'haphazard' non-systematic, single pair, polycrosses, nested and factorial designs.

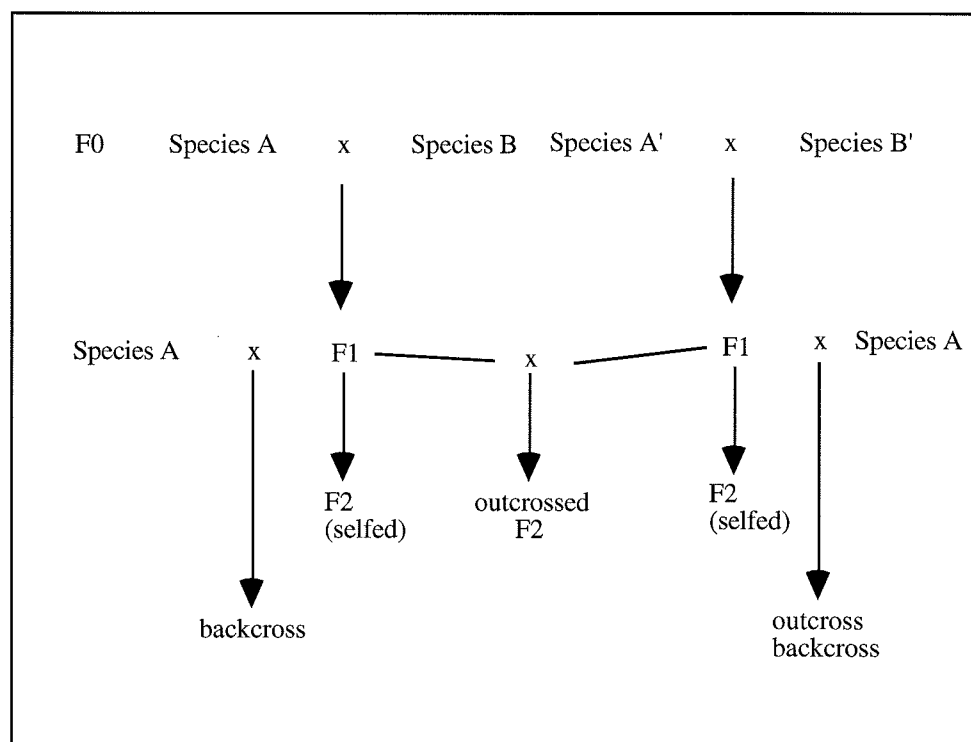


Fig. 5.2 Different type of interspecific hybrids produced by first and second generation crossing.

Some of the key, but most confused, terms in hybrid breeding are hybrid vigour and heterosis. As explained by Nikles and Griffin (1992) :-

" Some workers consider them synonyms while others use hybrid vigour to refer hybrid superiority without causal connotation, and heterosis to refer to superiority due to non-additive gene effects."

Superior hybrid performance may also result from additive gene effects where two traits showing predominantly additive inheritance complement each other, which is allied to the 'adaptiveness' concept used

by (Martin 1989). Such complementarity appears to be shown in the *E. urophylla* x *E. grandis* hybrid planted in Brazil where the hybrid gains resistance to stem canker from *E. urophylla* and the fast growth and form from *E. grandis*. Thus hybrid superiority may result from true heterosis (dependent on non-additive gene effects) or, as is more likely the case in forest trees, be due to the beneficial combination of complementary traits (dependent on additive gene effects). In the latter case, hybridisation of species with complementary traits may result in synergistic effects in specific environments where neither of the parents are well adapted. It is thus preferable to use the term hybrid vigour to refer to the superior performance of the hybrid without a causal connotation. Hybrid superiority may be measured in relation to the mean value of the two parents (i.e. the mid-parent value) or to the better or worse parent. The basis of comparison must always be clearly specified, but for operational use, performance relative to the better parent is most appropriate. Regardless of the cause, the expression of hybrid superiority will depend on the environment in which the hybrid is grown. For example, in environments where canker disease does not exist, the superiority of the *E. urophylla* x *E. grandis* over *E. grandis* may be diminished. It is thus also important to define the site or environment in which hybrid superiority is being assessed. Many of the successful hybrids report by (Martin 1989) are utilised in environments where neither of the parents are well adapted.

The other major issue in hybrid breeding is the base to which hybrid superiority is assessed (Potts *et al.* 1992, Sedgley and Griffin 1989). The first generation F₁ hybrid is always outcrossed whereas open pollinated control families of the parents may often be inbred to an unknown degree. In order to draw any general conclusion on the superiority of the hybrids it is necessary to use outcrossed parental controls. One of the causes of hybrid superiority may thus simply be a release from inbreeding which could just as easily be achieved through crossing within species. The release from inbreeding is believed to account for many of reports of hybrid superiority in eucalypts as the hybrids are only compared to open-pollinated controls or inbred land races (Sedgley and Griffin 1989).

There is a large array of potential species which may be crossed within the subgenera of *Eucalyptus*, and (Nikles and Griffin 1992) proposed potentially useful interspecific combinations could be identified by:-

- (i) their complementarity of traits
- (ii) fairly close taxonomic-genetic affinity
- (iii) target environments for planting which are intermediate between those in which the parental species are best adapted, and
- (iv) use the 'best' provenances and individuals.

Barriers to hybridisation

A detailed knowledge of crossability patterns is essential as reproductive barriers may preclude many otherwise desirable species combinations from direct commercial exploitation. The major barriers to the production of hybrids can be grouped into the categories indicated in Table 5.2. In the case of manipulated hybridisation, pre-mating barriers such as flowering time are simply overcome by pollen storage and the barriers of concern are mainly post-mating.

Table 5.2 Reproductive barriers in plants

- 1. Pre-mating barriers**
(e.g. flowering time, pollinators)
- 2. Post-mating**
 - 2a Pre-zygotic barriers**
(e.g. flower structure, barriers in stigma or style)
 - 2b Post-zygotic barriers**
(e.g. embryo abortion)
- 3. Post-dispersal barriers**
 - 3a F1 vigour, sterility**
 - 3b Advanced generation breakdown**

The major subgenera are reproductively isolated, as physiological barriers result in most pollen tube growth being arrested in the upper style (Ellis *et al.* 1991) (Fig. 5.4). However hybridisation within subgenera is relatively common (Pryor 1976; Griffin *et al.* 1988) and, in many cases, controlled pollinations amongst closely related species (within Section) have demonstrated few post-mating, pre-zygotic barriers to seedset (Cauvin 1983a; Cauvin *et al.* 1987; Potts and Cauvin 1988; Tibbits 1989). Nevertheless, two major pre-zygotic barriers to hybridisation within subgenera have been identified one which is structural the other physiological.

Structural

The structural barrier is unilateral and is due to the pollen tubes of small flowered species being unable to grow the full length of the style of large flowered species (e.g. Potts 1988; Gore *et al.* 1990). This structural barrier to hybridisation is encountered when trying to cross *E. globulus* and *E. nitens* which while relatively closely related differ markedly in flower size. The only way that the cross can be successfully produced is by using *E. nitens* as the female parent, as the pollen tubes of *E. nitens* only grow to about half the length of the large *E. globulus* style (Fig. 5.3).

There is considerable interest in breaking this structural barrier as the *E. globulus* flower is relatively large, is easy to pollinate and emasculate, and has the potential to produce larger numbers of seed than an *E. nitens* flower (Potts *et al.* 1992). However, treatments such as shortening the style of *E. globulus* (e.g. Fig. 5.4) have to date been unsuccessful.

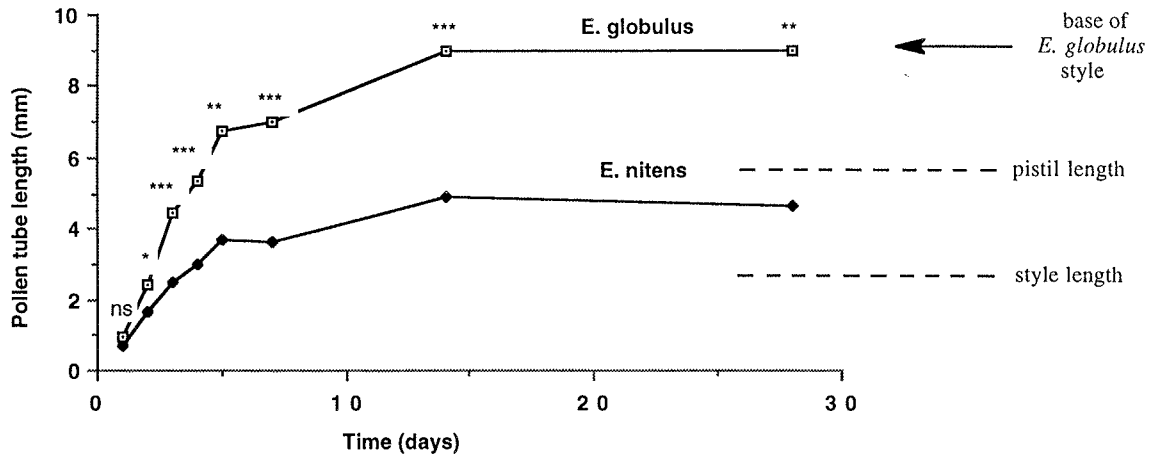


Fig. 5.3 The growth of *E. globulus* and *E. nitens* pollen tubes down the style of *E. globulus*.

The length of the *E. nitens* style and pistil are indicated. (Gore *et al.* 1990)

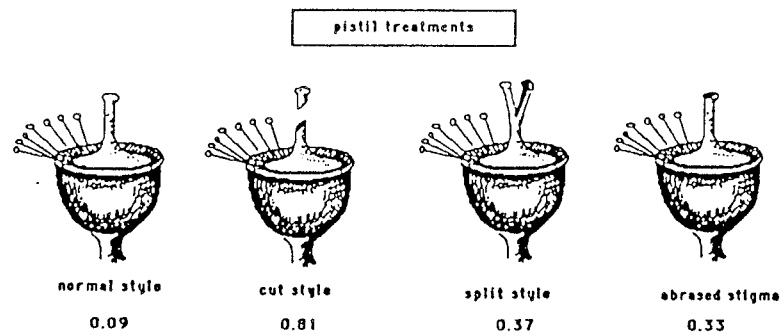


Fig. 5.4 Pistil treatments undertaken on *E. gunnii* flowers at anthesis and treated with *E. ovata* pollen (from Potts and Cauvin 1988).

Physiological barriers (incongruity)

There is mounting evidence to suggest that both pre-zygotic (Ellis *et al.* 1991) and post-zygotic (Pilipenko 1969; Potts *et al.* 1987; Griffin *et al.* 1988) barriers to crossing within subgenera increase with increasing taxonomic distance between species. The work of Ellis *et al.* (1991) has shown that if flower size is kept constant, then the severity of pollen tube abnormalities and the probability of pollen tube arrest in the pistil increased with increasing taxonomic distance between the parents. Some attempts to overcome barriers between incompatible pairs of species (e.g. to cross species from *Symphyomyrtus*, section *Transversaria* with those from the section *Adnataria*) have been successful by hexane treatment of the stigma (Pryor and Willing 1974) which implies a physiological basis to the barriers.

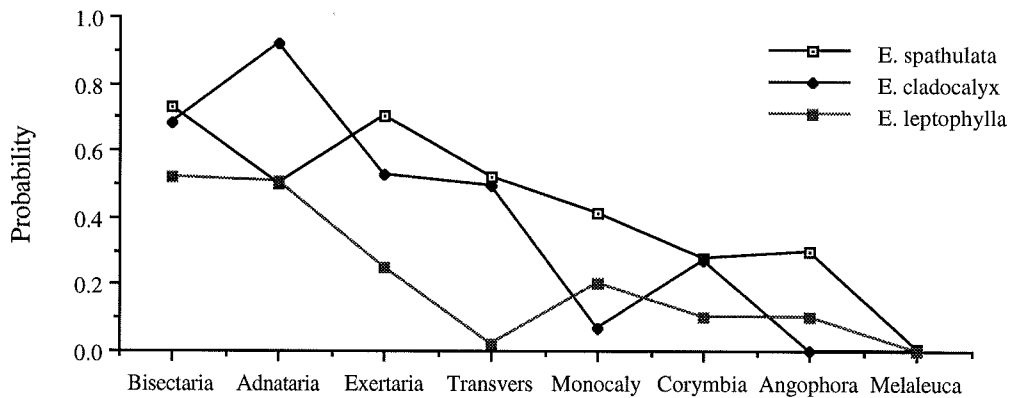


Fig. 5.5 The effect of taxonomic distance on the probability of pollen tubes reaching the base of the style in three species from the subgenus *Symphyomyrtus* section *Bisectaria* (Ellis *et al.* 1991).

Griffin *et al.* (1988) noted that while successful intersectional crosses have been made in the subgenus *Symphyomyrtus*, there was an increased frequency of viability problems in some cases, although there is no doubt that some intersectional crosses have yield quite successful hybrids (e.g. *E. grandis* x *tereticornis*). A study of hybrids with *E. gunnii* suggested that the performance of interspecific F₁ hybrids decreased in interseries crosses (Potts *et al.* 1987). An optimum degree of genetic/taxonomic divergence for cross success and the expression of hybrid vigour has been hypothesized (e.g. Potts *et al.* 1987; Martin 1987) resulting

Hypothetical relationship between cross success and the genetic distance between parents

(modified from Martin 1987)

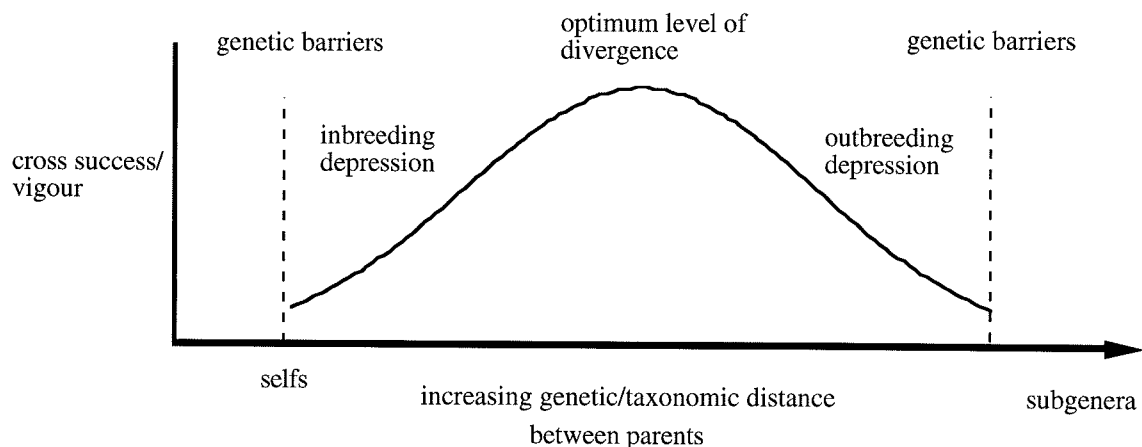


Fig. 5.2 Hypothetical relationship between cross success/vigour and the genetic distance between parents (Martin 1989).

from the expression of inbreeding depression at one extreme and outbreeding depression in wide interspecific crosses at the other.

Even in crosses between relatively closely related species high levels of abnormal individuals of poor vigour may occur, and often we have found that F₁ hybrid families may be quite variable with some families producing plants of extremely poor vigour and abnormal phenotypes whereas other F₁'s in the same family may be relatively vigorous. In the case of hybrids between *E. nitens* and *E. globulus*, high levels of abnormalities were reported in the nursery and field trials, although a high percentage of the hybrids were of normal phenotype (Potts *et al.* 1992; Volker 1995; Espejo *et al.* 1995). In the case of the *E. nitens* \times *E. globulus*, the performance of the F₁ hybrids was not predictable on the basis of parental performance in pure species combination (Volker 1995). There was also no correlation between parental performance in pure (GCA) and hybrid (GHA) combination (Volker 1995; see also Dieters *et al.* 1995). Barriers to hybridisation may extend beyond the F₁ generation and recombination and segregation in the F₂ may result in advanced generation hybrid breakdown (e.g. genomic incompatibility). However it is important to differentiate advanced generation hybrid breakdown from effects of inbreeding which are often confounded in advanced generation hybrids. In the case of *E. gunnii* \times *E. globulus* for example, high levels of semi-lethal abnormal phenotypes were observed in an F₂ family derived from selfing an F₁ hybrid, but not in an outcross of the same F₁ hybrid female to an unrelated F₁ (Vaillancourt *et al.* 1995). Molecular mapping and analysis of other cross types suggested that the abnormality was an effect of inbreeding, resulting from the expression of a deleterious recessive allele derived from the *E. gunnii* female (Vaillancourt *et al.* 1995).

6. Techniques for controlled pollination

Manipulation of pollen

Flower bud collection

Stems bearing flowers that are close to shedding their operculum (anthesis) are selected and cut to a length of about 30 - 50 cm. The open flowers (post anthesis) are removed to reduce the possibility of contamination, and the lamina are cut just above the petiole. The branches are labelled and enclosed in plastic bags to eliminate contamination between pollen lots. In the laboratory, the stems are placed in a vessel and cut under water.

Dry Pollen extraction

The methods we have found to be effective for the extraction and collection of pollen which is not sticky (e.g. *E. globulus* and *E. nitens*) are simple, inexpensive and produce pollen which should remain viable for at least 12 months when frozen. If care is taken, the pollen will be free from staminal tissue and other debris.

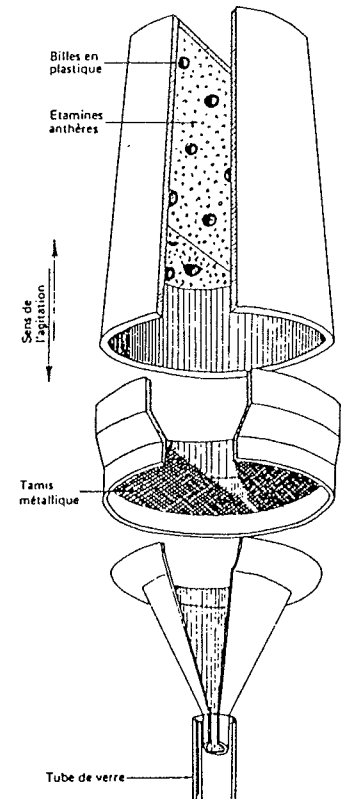
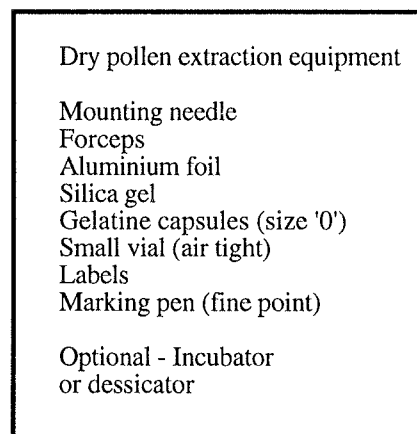
When the operculum is shed (anthesis) the filaments are inflexed (Fig. 6.1). The flowers are left until the filaments unfold (approximately 24 - 48 hours), at which time the pollen is freely shed. The flowers (or just the staminal ring with stamen attached) are cut from the stem and placed onto aluminium foil made into an envelope shaped package. Care must be taken to ensure that no contamination occurs between pollens from different trees. The flowers are dried for 12-24 hours at approximately 25-30° C. An incubator is useful for the drying stage, or the flowers may be left in a desiccator over silica gel. Longer drying periods cause the stamens to become brittle and break, resulting in "unclean pollen". The pollen is shaken from the flower onto the foil by tapping or by holding the flower with forceps and rubbing the base of the stamens with a mounting needle. We store pollen in gelatine capsules ("o" size), with each gelatin capsule containing sufficient pollen for use in a single crossing session (i.e. approximately 1/4 of their total capacity). The pollen is scraped from the foil directly into a gelatin capsule. Partially filling the capsule minimises wastage, which may occur if the gelatin capsule deteriorates during field use. Each gelatin capsule is labelled with a fine point marking pen, and then all gelatin capsules from the same tree are placed together into a labelled glass vial (e.g. 8.5 x 2.5 cm) containing a small quantity of active silica gel. Gelatine capsules are extremely sensitive to moisture, softening rapidly, thus in the rain, care must be taken when using pollen stored with this method. The method described is particularly suited to *E. globulus* which has large flowers and produces abundant pollen.



Fig. 6.1 Flower bud just before emasculation (from van Wyk 1981b).

A variant of this method involves shaking the pollen through a 200 micron sieve inserted into a small plastic funnel (Fig. 6.2). The capsule is held on the end of the funnel, while the vessel is vigorously shaken. This method is particularly useful when extracting pollen from small flowers (e.g. *E. nitens*). When flowers are small and in short supply, the flowers themselves can be placed directly in a gelatin capsule and dried.

Fig. 6.2 Extraction of pollen using a sieve system (from Cauvin 1983)



References: (Cauvin 1983a), (van Wyk 1981b)

Wet extraction

A method of wet extraction of pollen which is described by Griffin *et al.* (1982) but is no longer used operationally due to its expense, requirement for specialised equipment and slowness. However, this method has been used in conjunction with a haemocytometer to make mixes of selfed and outcrossed pollen of known proportions (Sedgley *et al.* 1989) and has potential in research. It may also be useful for species which have sticky pollen.

Anthers are clipped from flowers with scissors and pollen extracted using a double filtration procedure. The anthers are placed in a glass tissue grinder with 5 ml of distilled water, and then ground with a pestle. The

resulting homogenate is filtered through a nylon cloth of 37 micron pore size. This allows the pollen grains to pass through while retaining most tissue debris. About 100 ml of water is needed to wash the pollen through this stage of filtration. The filtrate is then run through a Milipore filtration system with a 3 micron pore filter. A hand pump is used to partially evacuate the system and hasten removal of the filtrate. The filter plus deposited pollen is finally removed to dry in a desiccator over silica gel. If the pollen is to be used for controlled pollination work the filter should be backed with an adhesive label, as it can become rather brittle on drying.

For storage and subsequent use in pollination it is convenient to cut the filter into segments and store these in small (10 ml) plastic capped bottles. These can be carried into the field and segments extracted as required for use. The broad end of a segment which does not carry pollen may be grasped with the fingers or forceps allowing application of pollen to receptive stigmas. The pollen cakes onto the filter and therefore does not fall off, so pollination can be carried out at any angle. The act of rubbing onto a stigma will generally remove more than sufficient pollen grains, but if desired the face of the caked pollen can also be loosened by scraping gently with a scalpel blade.

Pollen storage

Eucalypt pollen is quite robust but freezing (-18°C to -20°C) is required to maintain pollen viability is retained over long time intervals. In the short term (< 1 month) pollen may be stored at room temperature or more preferably in a fridge, without a significant reduction in viability. Silica gel is added to vials to eliminate pollen rehydration. Freeze Drying pollen may increase its longevity, however the freeze drying equipment is expensive.

References: (Boden 1958), (van Wyk 1977), (Griffin, et al. 1982), (Potts and Marsden-Smedley 1989)

Testing pollen viability

Pollen viability tests are carried out when pollen is first collected and before pollen is used for controlled pollinations. As viability declines over time, the importance of testing pollen becomes greater with increasing storage time. The exact level to which viability must drop before the cross success is affected has not been determined. While pollen staining has been used by several authors, the relative ease with which eucalypt pollen is germinated *in vitro* has made this approach unnecessary. Most *in vitro* germination is undertaken using either liquid or a semi-solid agar media.

In vitro techniques

Liquid medium

A successful liquid media, consisting of 30 percent sucrose and 150 ppm boric acid is autoclaved for 10 minutes at 69 KPa. The solution can be frozen for later use. After thawing in a microwave, approximately 2 ml of the media is transferred into a vial. A toothpick is used to transfer a small amount of pollen from the capsule into the vial. The vials are shaken, labelled and incubated for 24 hours at 30°C . A pipette is used to transfer a drop of solution onto a glass microscope slide. A cover glass is placed on the drop and the slide viewed at 100X magnification. The percentage of germinated grains is determined by counting. For practical purposes, a grain is usually considered germinated if the pollen tube is $1/3$ the diameter of the pollen grain. This technique is not appropriate if pollen tube length is of interest as breakage of the pollen tubes may occur.

Liquid pollen germination media

1 litre of medium

- 300 g sucrose (30%)
- 150 mg boric acid (150 ppm)
- make to volume with distilled water, autoclave

Equipment

- Small vial
- toothpick
- Light microscope (100X)
- glass slides
- cover slips
- autoclave
- incubator

Incubate at 30°C for 24 hours
Glass slides and cover slips
Score 100x magnification

References: (Griffin *et al.* 1982)

Agar medium

Agar media, consisting of 20% sucrose, 150 ppm boric acid and 0.5% agar is autoclaved for 10 minutes at 69 Kpa. The solution is plated into cells in 5X5 replidishes or small petridishes in a lamina flow cabinet. The dishes can be frozen for latter use. When they have thawed, scrap a small amount of pollen out of the gelatin capsule and streak this across the surface of the media. Incubate at approximately 25-30° C for 24 hours and view at 100X magnification.

Agar pollen germination media

1 litre of medium

- 200 g sucrose (20%)
- 150 mg boric acid (150 ppm)
- 5 g agar (0.5%)
- make to volume with distilled water, autoclave

Equipment

- toothpick
- replidish (or petri dish)
- autoclave
- incubator
- Light microscope (100X)

Incubate at 25 - 30°C for 24 hours
Score 100x magnification

Detailed study of the response of germinating eucalypt pollen on variation in boric acid and sucrose concentrations in the agar mix undertaken by Potts and Marsden-Smedley (1989) have indicated that while the concentration of sucrose for optimal pollen tube growth is less than that for optimal germination, 20% sucrose is not significantly different from optimal for both traits (Potts and Marsden-Smedley 1989).

References: (Boden 1958), (Cauvin 1983a), (Heslop-Harrison and Heslop-Harrison 1985), (Potts and Marsden-Smedley 1989).

In vivo techniques

Germination of pollen can be also determined by examining the frequency and growth of pollen tubes in styles pollinated with the test pollen.

Method 1 (modified from Griffin *et al.* 1982)

1. Fix freshly cut styles in a 1:3 acetic acid/ ethanol solution for 1-3 hours, after which they may be stored indefinitely in 70% ethanol.
2. Rinse style in distilled water, then place on a glass slide under a dissecting microscope, slit the epidermis longitudinally using a scalpel. Care should be taken to avoid cutting deeply into the transmission tissue.
3. Autoclave for 10 minutes at 120°C in an aqueous sodium sulphite solution (50g/ litre) to soften the tissues. Rinse free of sodium sulphite with distilled water.
4. Remove the epidermis under a dissecting microscope with forceps. It is possible to simply peel the epidermis away at this stage.
5. Stain with 0.1% analine blue in 0.1 M tri-potassium ortho-phosphate including 10% glycerine, which has been decolourised by storing in darkness for 24 hours prior to use. This fluorochrome is used because of its specificity for the callose deposited in pollen tubes.
6. Place a cover slip over dissected styles, and a second glass slide over that; squash with minimal lateral movement and remove upper slide. If desired seal preparation with clear nail polish.
7. Observe using a reflected light fluorescence microscope. A total magnification of about 80x is suitable for general scanning, and 200x for detailed observation and counting of numbers of pollen tubes per style .

A time saving variation of this method by Griffin *et al.* (1982), involves eliminating steps 2, 3 and 5, and cutting transverse sections of the style by hand. It is helpful to use a small piece of carrot, with a slit down one side, to hold the style in place. Using a blade, cut thin section and stain as in step 4. Place the sections on a glass slide and position a cover slip over them. View as in step 6.

Fluorescent microscopy (Method 1)

1:3 acetic acid/ ethanol solution
70% ethanol
distilled water
50g/litre sodium sulphite solution
0.1% analine blue stain

fluorescent microscope
autoclave
glass slides and cover slips

Method 2 (from Sedgley and Smith 1989)

Pistils or styles are harvested. A longitudinal slit is placed in the styles to sever the thick cuticle. If ovules are required, these can also be dissected from the locules and both ovules and the styles, fixed in Carnoy's fixative (6:1:3 absolute ethanol: glacial acetic acid: chloroform) for a minimum of 24 hours and stored at 4°C. The material is then hydrated through an ethanol series, softened in 0.8N NaOH at 60°C for up to one hour, stained in decolourised analine blue (overnight) and then squashed in 80% glycerol and viewed using fluorescent microscopy. Styles are squashed on the slides with the longitudinal incision uppermost to ensure that the thick cuticle lay underneath the pollen tubes and does not obscure their observation and counting. Ovules are mounted on separate slides from the styles as they require less squashing.

Fluorescent microscopy
(Method 2)

Carnoy's fixative

- absolute ethanol 60 ml
- glacial acetic acid 10 ml
- chloroform (pure) 30 ml

ethanol series for hydration

0.8 N NaOH

50g/litre sodium sulphite solution

decolourised aniline blue

80% glycerol

fluorescent microscope

glass slides and cover slips

References: (Griffin and Hand 1979), (Sedgley *et al.* 1989), (Sedgley and Smith 1989), (Gore *et al.* 1990), (Ellis 1991)

Controlled pollination

Emasculation

Flowers are emasculated when the operculum begins to change to a yellow colour and starts to lift away from the receptacle (Fig. 6.1). At this stage the pollen has generally not been released from the anthers. Emasculation involves cutting away the staminal ring so as to remove all the stamens, with minimal tissue damage. Modified electricians pliers are generally used on small flowered species such as *E. nitens*, or a scalpel on large flowered species such as *E. globulus*. It is common for some filaments to break, leaving anthers lodged around the edge of the disc. These can be removed either by vigorous blowing or using the tip of an obliquely cut petiole to push them out. Care must be taken to avoid injuring the stigma, style and or the disc, as injury may lead to flower abortion (probably due to infection by pathogens). Unemasculated flowers are removed using secateurs. It is important to synchronise the developmental stage of the flowers within a bag as closely as possible so that all flowers will be receptive at a similar time.

References: Meskimen (1965), (Hodgson 1976b), (van Wyk 1977), (Cauvin 1983b), (van Wyk 1981b), (Potts and Potts 1986)

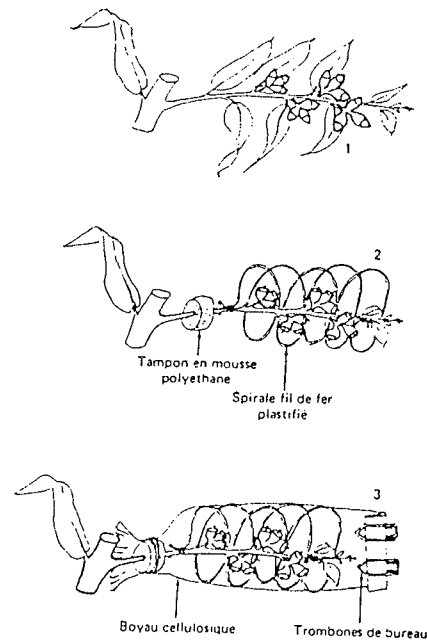
Isolating flowers

Flowers are isolated to prevent natural pollination. The branches bearing buds are often pruned, removing leaves (or more often about one half of the lamina) and small stems, to fit inside the bags. Leaves on the ends of the branches are left intact. Bags are identified by tying a durable label to the branch immediately above them. Aluminium labels (eg. Dymo tape) are ideal for this purpose.

Celluloid bags

Celluloid bags are supported by a spiral wire frame (Fig. 6.2). The wire frames are cut to length and taped to the branch, and the celluloid bag pulled over the frame. Plumbers insulation can be taped to the

Fig. 6.2 Construction of a wire frame for supporting pollination bags
(from Cauvin 1983b).



branch to provide a more secure closure of the bag on the branch. A small slit is cut in the bag to allow pollination, and the hole is sealed with tape after pollen application. Electrical ties are used to close the ends of the bag. The bags can be reopened by cutting the ties with wire cutters. While bags can completely enclose the growing end of the branch (e.g. Fig. 6.2), it is better to choose branches which will allow the protrusion of the growing end of the bag as shown in Fig. 6.3. This allows healthy growth to continue and alleviates the necessity to remove the bag early.



Fig. 6.3 Celluloid pollination bag with spiral wire frame
(from Potts and Potts 1986).

Terylene bags

Pollination bags made from non-woven polyester, Terylene, with a PVC window on the front are durable, and while relatively expensive, they can be reused for many years. The majority of the leaf material to be enclosed within the bag is removed, generally by cutting the lamina in half. Wire frames are generally not used, but when enclosing flowers care is taken to avoid having them touching the bag. Slightly inverting the corners of the bags helps to keep them expanded. The ends of the bags are opened and closed as described above.

Supplier:
 PBS International
 Salter Road, Eastfield Industrial Estate,
 Scarborough, YO11 3UZ
 N. Yorkshire, England.

Style isolation

Recent developments in controlled pollination techniques have included a method of isolating individual styles. This is achieved using either rubber tubing or plastic drinking straws with a bore diameter of 3 - 6 mm, which are set in glue applied to the disc. The top of the tube is then closed off with a staple or cotton wool to isolate the style, and latter cut open to allow pollen application. Direct style isolation saves time, as the isolating tube can be left in place on the developing fruit, whereas conventional isolation techniques using pollination bags require debagging .

Post-anthesis floral development

Timing of receptivity

Morphological changes

Controlled pollination efficiency is to a large extent dependant on the synchronous timing of pollen application with stigma receptivity. Morphological changes are the best guide for timing articial pollination. Stigma receptivity is indicated by a flattening of the stigma and the production of a sticky exudate (Moncur and Boland 1989; Griffin and Hand 1979), and in some species, style length increases (e.g. *E. regnans*, Griffin and Hand 1979). Stigma receptivity has also been correlated to a positive estase test (Anderson 1984; Savva *et al.* 1988), but visual observation is usually sufficient in most cases (Figs 3.1, 3.2 and 6.4).

The exact timing of the onset of stigma receptivity varies with species (Table 3.2; Fig. 3.2). The time between operculum lift and the onset of stigma receptivity for *E. globulus* and *E. nitens* is 5-8 days, although this can vary with the intervening weather conditions and site, with cold temperatures delaying receptivity (Griffin and Hand 1979; Hodgson 1976b). Whether pollen applied prior to receptivity will remain attached to the stigma and viable may depend upon the species, and it is possible the pin-head or blunt style of *Symphyomyrtus* species is better at holding pollen during the non-receptive phase than the hollow style of *Monocalyptus* species such as *E. stellulata* and *E. regnans* (Griffin and Hand 1979). The stigma of most species seems to remain receptive for four or five days.

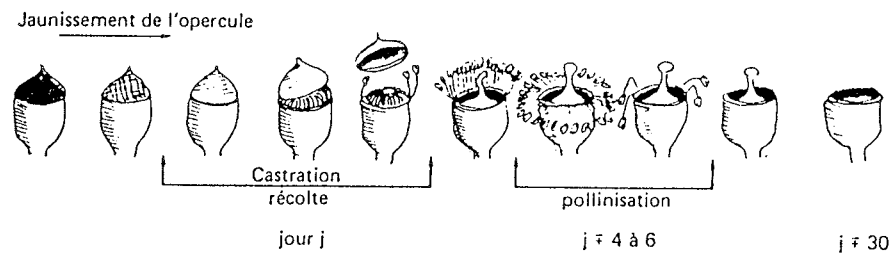


Fig. 6.4 Post anthesis development of the flower of *E. gunnii* showing the periods for emasculation and pollination (from Cauvin 1983b).

In vivo pollen germination

In vivo pollen germination tests can be used to determine the precise timing of stigma receptivity. Select and label approximately three to five flower buds at anthesis, each day, over a period of two weeks. Further, on a daily basis apply pollen to each of the flowers. At the end of the two week period, harvest the buds and process by the method outlined by Griffin *et al.* (1982) (see *In vivo* technique: pollen tube growth in the style). Using the labelled sequence, determine the number of days post anthesis to stigma receptivity based on observations of germinating pollen grains.

Reference: (Ellis 1991)

Pollination techniques

Gelatin capsules containing viable pollen are placed in small air-tight vials for field work. There are a variety of ways of applying the pollen to the receptive stigma. Pollen can be applied to the receptive stigmas using a toothpick or match, or small, fine paint brush. If paint brushes are used they must be washed in 70% ethanol and dried thoroughly before re-use with a different pollen. We prefer to simply use a toothpick or match as these can be discarded after each application. When matches are used we prefer 'redheads' as the pollen is clearly visible on the head of the match, but when the matchhead starts becoming moist it is discarded. The isolation bags are temporarily re-opened for pollination and quickly re-closed. In the case of celloid bags, a small opening may be cut in the bag which is then re-sealed with electricians tape. This is quicker and avoids re-opening and sealing the bag ends.

Pollen can also be applied by 'anther' using either frozen or freshly collected flowers (van Wyk 1977 and 1981b). A branch with flowers about 2 days post anthesis is simply cut, transported directly to the females and single flowers picked as required. The pollen is applied by rubbing the anthers on the stigma of the flower to be pollinated. The pollen flower must be handled carefully to avoid nectar contaminatin of the pollen which reduces the efficiency of pollination.

Timing of pollen application	
Species	Number of days after anthesis
<i>E. globulus</i>	4-8
<i>E. gunnii</i>	5-6
<i>E. nitens</i>	approx. 7
<i>E. obliqua</i>	4-5
<i>E. regnans</i>	10-14

References: (Griffin and Hand 1979), (Cauvin *et al.* 1987), (Potts and Potts 1986), (Tibbits 1989), (Moncur and Boland 1989).

Self pollinations

Assisted and unassisted self pollinations are equally effective in *E. globulus* (Potts and Cauvin 1988). Because of the protandrous nature of eucalypts, when unassisted self pollinations are performed several flowers are enclosed in each bag. The flowers can be isolated well before anthesis. Assisted self pollinations can be performed by manual application of pollen, or insect assisted pollen application. In the latter case fly pupae (Pryor and Boden 1962) are enclosed in the isolation bag at a time that allows their emergence as adults to coincide with stigma receptivity. Self pollination results in reduced seed set (Potts and Savva 1988), therefore large numbers of crosses are required to produce the number of seed desired.

Debagging

Isolation bags are removed about 4 - 8 weeks after pollination, at which time an abscission layer is developing at the base of the styles. By about 8 weeks, unsuccessful crosses will abort. Branches bearing young fruit are labelled with brightly coloured flagging tape to aid in locating the mature fruit at harvest time.

Collection of fruit and seed extraction

Mature fruit are collected approximately one year after pollination. It is advisable to collect open pollinated fruit prior to harvesting controlled pollinated fruit, to test seed maturity/germination. The fruit are placed into clearly labelled envelopes, individually if seed set data is required. These are stored in a warm dry environment until the valves have fully opened. The seed is extracted by vigorously shaking the fruit in a vessel / sieve and in the case of controlled crosses it is worth while gently probing the inside of the capsule with a dissecting needle to dislodge basal seed. The contents can be classified into one of the following categories- viable seed, inviable seed, squashed, chaff and insect attacked. Viable seed are large, plump and dark coloured, while inviable seed appear sunken or flat, but generally dark coloured. Marginal cases are squashed and structures with a firm white embryo are classified as viable/squashed. In some species capsules developed from emasculated flowers may prematurely open their valves while still green on the tree due to damage caused by emasculation. In such cases regular checking of maturing capsules is advisable to prevent loss of seed from capsules opening or drying out the tree.

Parthenocarpic fruit development has been reported in *E. grandis* (Hodgson 1976c), but not in other species examined such as *E. regnans* (Griffin and Hand 1979) and mature capsules containing no viable seed are rare in species such as *E. nitens* (Tibbits 1989) and *E. globulus* (Potts and Savva 1989).

Checklist of Controlled Pollination Equipment

Emasculator- Scalpel, Scalpel blades, Modified electricians pliers
 Isolation bags - Terylene bags or Celluloid casing
 Coil wire and bending tube
 Wire cutters
 Plumbers insulation
 Staples and stapler
 Secateurs
 Electrical cable ties
 Electricians tape (sticky)
 Aluminium labels
 Tie wire
 Flagging tape
 Note book
 Pencil
 Pollen
 Pollen applicator (matches, fine paint brush)
 Wire cutters
 Hand magnifying lens
 Ladder
 Rope
 Hat
 Sunscreen lotion
 Band aids

Location :

[illegible]

A circular diagram with a vertical line and a horizontal line intersecting at the center. The vertical line is labeled 'North' at the top and 'South' at the bottom. The horizontal line is labeled 'West' on the left and 'East' on the right.

Bibliography

- Anderson, C.A. (1984). The anatomy and histochemistry of the pistil of *Eucalyptus obliqua* L'Herit. In 'Pollination '84', The University of Melbourne.
- Ashton, D.H. (1975). Studies of the flowering behaviour in *Eucalyptus regnans* F. Muell. *Australian Journal of Botany* **23**, 399-411.
- Barber, H.N. (1965). Selection in natural populations. *Heredity* **20**, 551-572.
- Barlow, B.A. (1981). The Australasian Flora: Its origin and evolution In 'Flora of Australia.' (Ed. R. Robertson, B.G. Briggs, H. Eichler, L. Pedley, J.H. Ross, D.F. Symon and P.G. Wilson) pp. 25-75 (Australian Government Publishing Service: Canberra.)
- Blakely, W.F. (1934). 'A Key to the Eucalypts.' (The Worker Trustees: Sydney.)
- Boden, R.W. (1958). Handling and storage of pollen in *Eucalyptus* breeding. *Australian Forestry* **12**, 73-81.
- Boland, D.J., Brooker, M.I.H., Chippendale, G.M., Hall, N., Hyland, B.P.M., Johnston, R.D., Kleinig, D.A. and Turner, J.D. (1985). 'Forest Trees of Australia.' (Australian Government Publishing Service: Melbourne.)
- Boland, D.J., Brooker, M.I.H., Turnbull, J.W. and Kleinig, D.A. (1980). 'Eucalyptus seed.' (CSIRO: Melbourne.)
- Boland, D.J. and Sedgley, M. (1986). Stigma and style morphology in relation to taxonomy and breeding systems in *Eucalyptus* and *Angophora* (Myrtaceae). *Australian Journal of Botany* **34**, 569-584.
- Bolotin, M. (1975). Photoperiodic induction of precocious flowering in a woody species *Eucalyptus occidentalis* Endl. *Botanical Gazette* **136**, 358-365.
- Brooker, M.I.H. and Kleinig, D.A. (1983). 'Field Guide to Eucalypts South-eastern Australia.' (Inkata Press: Melbourne.)
- Carr, S.G.M. and Carr, D.J. (1962). Convergence and progression in *Eucalyptus* and *Symphyomyrtus*. *Nature* **196**, 969-972.
- Cauvin, B. (1983a). 'Eucalyptus hybridation controlee. Premiers resultats. Annales de Recherches Sylvicoles, AFOCEL, 85-117.'
- Cauvin, B. (1983b) Précautions pour l'hybridation contrôlée des *Eucalyptus*. In 'Colloque international sur les *Eucalyptus* résistants au Froid', Bordeaux, France. (AFOCEL). pp 509-522
- Cauvin, B., Potts, B.M. and Potts, W.C. (1987). *Eucalyptus* : Hybridation artificielle - barrières et hérédité des caracteres In 'Annales de recherches sylvicoles.' pp. 255-303 (Association Forêt-Cellulose: Paris.)
- Chippendale, G.M. (1988). 'Eucalyptus, Angophora (Myrtaceae). Flora of Australia 19.' Vol. 19 (Australian Govt. Publishing Service: Canberra.)
- Churchill, D.M. (1968). The distribution and prehistory of *Eucalyptus diversicolor* F. Muell., *E. marginata* Donn ex Sm., and *E. Calophylla* R.Br. in relation to rainfall. *Australian Journal of Botany* **16**, 125-151.
- Cremer, K.W. (1965a). Effects of fire on seedshed from *Eucalyptus regnans*. *Australian Forestry* **29**, 252-262.
- Cremer, K.W. (1965b). How eucalypt fruits release their seed. *Australian Journal of Botany* **13**, 11-16.
- Cremer, K.W. (1977). Distance of seed dispersal in eucalypts estimated from seed weights. *Australian Forest Research* **7**, 225-228.
- Davis, G.L. (1968). Floral morphology and the development of gametophytes in *Eucalyptus melliodora* A. Cunn. *Australian Journal of Botany* **16**, 19-35.

- Davis, G.L. (1969). Floral morphology and the development of gametophytes in *Eucalyptus stellulata* Sieb. *Australian Journal of Botany* **17**, 177-190.
- Dieters, M. J., Nikles, D.G., Toon, P.G. and Pomroy, P. (1995). Hybrid superiority in forest trees - concepts and application. In 'Eucalypt Plantations, Improving Fibre Yield and Quality' (eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond). pp. 152-155. Proc. CRCTHF-IUFRO Conf., Hobart, 19-24 Feb. (CRC for Temperate Hardwood Forestry: Hobart).
- Drake, D.W. (1975). Seed abortion in some species and interspecific hybrids of *Eucalyptus*. *Australian Journal of Botany* **23**, 991-995.
- Drinnan, A.M. and Ladiges, P.Y. (1989). Operculum development in *Eucalyptus cloeziana* and *Eucalyptus* informal subg. *Monocalyptus* (Myrtaceae). *Plant Systematics and Evolution* **166**, 183-196.
- Drinnan, A. N. and Ladiges, P. Y. (1991a). Floral development and systematic position of *Eucalyptus curtisii* (Myrtaceae). *Australian Systematic Botany* **3**, 1-8.
- Drinnan, A. N. and Ladiges, P. Y. (1991b). Floral development in the 'Symphyomyrtus group' of eucalypts (Myrtaceae). *Australian Systematic Botany* **4**, 539-551.
- Duncan, F. (1989). Systematic affinities, hybridisation and clinal variation within Tasmanian eucalypts. *Tasforests* **2**, 13-24.
- Eldridge, K.G. (1976). Breeding systems, variation and genetic improvement of tropical eucalypts. Tropical trees. Variation, breeding and conservation. In 'Linnean Soc. Symp. Series No. 2.' (Ed. J. Burley and B.T. Styles) pp. 101-108 .
- Eldridge, K., Davison, J., Harwood, C. and van Wyk, G. (1993). Eucalypt domestication and breeding. (Clarendon Press, Oxford).
- Eldridge, K.G. and Griffin, A.R. (1983). Selfing effects in *Eucalyptus regnans*. *Silvae Genetica* **32**, 216-221.
- Ellis, M.F. (1991). 'Breeding Systems and Interspecific Hybridisation in the Genus *Eucalyptus* L'Her.' Unpublished PhD Thesis, The University of South Australia.
- Ellis, M.F., Sedgley, M. and Gardner, J.A. (1991). Interspecific pollen-pistil interaction in *Eucalyptus* L'Hér. (Myrtaceae); the effect of taxonomic distance. *Annals of Botany* **68**, 185-194.
- Espejo, J.E., England, N.F. and Griffin, A.R. (1995). Results of a crossing program with *Eucalyptus nitens* and *E. globulus* in Chile. In 'Eucalypt Plantations, Improving Fibre Yield and Quality' (eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond). pp. 239-240. Proc. CRCTHF-IUFRO Conf., Hobart, 19-24 Feb. (CRC for Temperate Hardwood Forestry: Hobart).
- Florence, R.G. (1964). A comparative study of flowering and seed production in six blackbutt (*Eucalyptus pilularis* Sm) forest stands. *Australian Forestry* **28**, 23-33.
- Foster, A.S. and Gifford, E.M. (1974). 'Comparative Morphology of Vascular Plants.' (W. H. Freeman and Company: San Francisco.)
- Frenguelli, J. (1953). Restos del genero *Eucalyptus* en el Mioceno de Neuquen. *Notas Museo Planta* **16**, 209-213.
- Fripp, Y.J., Griffin, A.R. and Moran, G.F. (1987). Variation in allele frequencies in the outcross pollen pool of *Eucalyptus regnans* F. Muell. throughout a flowering season. *Heredity* **59**, 161-171.
- Gaub, E. and Pryor, L.D. (1958). Seed coat anatomy and taxonomy in *Eucalyptus*. I. *Proceedings of the Linnean Society of New South Wales* **83**, 20-31.
- Gore, P. and Potts, B. M. (1995). The genetic control of flowering time in *Eucalyptus globulus*, *E. nitens* and their F₁ hybrids. In 'Eucalypt Plantations, Improving Fibre Yield and Quality' (eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond). pp. 241-242. Proc. CRCTHF-IUFRO Conf., Hobart, 19-24 Feb. (CRC for Temperate Hardwood Forestry: Hobart).

- Gore, P.L., Potts, B.M., Volker, P.W. and Megalos, J. (1990). Unilateral cross-incompatibility in *Eucalyptus* : the case of hybridisation between *E. globulus* and *E. nitens*. *Australian Journal of Botany* **38**, 383-394.
- Griffin, A.R. (1980). Floral phenology of a stand of mountain ash (*Eucalyptus regnans* F. Muell.) in Gippsland, Victoria. *Australian Journal of Botany* **28**, 393-404.
- Griffin, A.R. (1982) Pollination ecology of eucalypts - a framework for study. In 'Pollination '82. Proceedings of the Symposium held at Melbourne University.', (Ed. E.G. Williams, R.B. Knox, J.H. Gilbert and P. Bernhardt) pp 42-56
- Griffin, A.R. (1989a). Sexual reproduction and tree improvement strategy - with particular reference to *Eucalyptus* In 'Breeding Tropical Trees: Population structure and genetic improvement strategies in clonal and seedling forestry. Proceedings of the IUFRO conference, Pattaya, Thailand, November 1988.' (Ed. G.L. Gibson, A.R. Griffin and A.C. Matheson) pp. 52-67 (Oxford, United Kingdom; Arlington, Virginia, USA: Oxford Forestry Institute; Winrock International.)
- Griffin, A.R. (1989b). Strategies for the genetic improvement of yield in *Eucalyptus* In 'Biomass Production by Fast-Growing Trees.' (Ed. J.S. Pereira and J.J. Landsberg) pp. 247-265 (Kluwer Academic Publishers)
- Griffin, A.R. (1990). Effects of inbreeding on growth of forest trees and implication for management of seed supplies for plantation programmes In 'Reproductive Ecology of Tropical Forest Plants.' (Ed. K.S. Bawa and M. Hadley) pp. 355-372 (UNESCO and The Parthenon Publishing Group: Paris, France.)
- Griffin, A.R., Burgess, I.P. and Wolf, L. (1988). Patterns of natural and manipulated hybridisation in the genus *Eucalyptus* L'Herit.-a review. *Australian Journal of Botany* **36**, 41-66.
- Griffin, A.R., Ching, K.K., Johnson, K.W., Hand, F.C. and Burgess, I.P. (1982). Processing *Eucalyptus* pollen for use in controlled pollination. *Silvae Genetica* **31**, 198-203.
- Griffin, A.R. and Cotterill, P.P. (1988). Genetic variation in growth of outcrossed, selfed and open-pollinated progenies of *Eucalyptus regnans* and some implications for breeding strategy. *Silvae Genetica* **37**, 124-131.
- Griffin, A.R. and Hand, F.C. (1979). Post-anthesis development of flowers of *Eucalyptus regnans* F. Muell. and the timing of artificial pollination. *Australian Forest Research* **9**, 9-15.
- Griffin, A.R., Moran, G.F. and Fripp, Y.J. (1987). Preferential outcrossing in *Eucalyptus regnans* F. Muell. *Australian Journal of Botany* **35**, 465-475.
- Haque, M.S. (1984). Chromosome morphology in 4 species of *Eucalyptus* L. Her. *Cytologia* No. 3.
- Hardner, C.M. and Potts, B.M. (1993) Some effects of selfing *Eucalyptus globulus*. In 'Research Working Group No. 1 of the Australian Forestry Council, Forest Genetics, Proceedings of Twelfth Meeting, Canberra; February 1993', Canberra. (Australian Forestry Council).
- Hardner, C. M. and Potts, B. M. (1994). Inbreeding depression and changes in variation after selfing *Eucalyptus globulus* subsp. *globulus*. *Silvae Genetica* (in press).
- Hardner, C. M. and Potts, B. M. (1995). The influence of mortality and stand age on the estimation of genetic parameters in *Eucalyptus regnans*. In 'Eucalypt Plantations, Improving Fibre Yield and Quality' (eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond). pp. 208-211. Proc. CRCTHF-IUFRO Conf., Hobart, 19-24 Feb. (CRC for Temperate Hardwood Forestry: Hobart).
- Hardner, C. M., Potts, B. M. and Gore, P. (1995). Relationship between cross success and the geographical proximity of *Eucalyptus globulus* subsp. *globulus* parents. In 'Eucalypt Plantations, Improving Fibre Yield and Quality' (eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond). pp. 243-244. Proc. CRCTHF-IUFRO Conf., Hobart, 19-24 Feb. (CRC for Temperate Hardwood Forestry: Hobart).

- Hasan, O. and Reid, J.B. (1995). Reduction of generation time in *Eucalyptus globulus*. Plant Growth Regulators (in press).
- Heslop-Harrison, J. and Heslop-Harrison, Y. (1985). Germination of stress-tolerant *Eucalyptus* pollen. *Journal of Cell Science* **73**, 135-157.
- Heslop-Harrison, Y. and Shivanna, K.R. (1977). The receptive surface of the angiosperm stigma. *Annals of Botany (London)* **41**, 1233-1258.
- Hill, R. S. (1994). The history of selected Australian taxa. In 'History of the Australian vegetation: Cretaceous to Recent' (Ed. R. S. Hill). pp. 390-419. (Cambridge University Press; Cambridge.)
- Hodgson, L.M. (1976a). Some aspects of flowering and reproductive behaviour in *Eucalyptus grandis* (Hill) Maiden at J. D. M. Keet forest research station: 3. Relative yield, breeding systems, barriers to selfing and general conclusions. *South African Forestry Journal* **99**, 53-58.
- Hodgson, L.M. (1976b). Some aspects of flowering and reproductive behaviour in *Eucalyptus grandis* (Hill) Maiden at J.D.M. Keet forest research station (formerly Zomerkomst forest research station) 1. Flowering, controlled pollination methods, pollination and receptivity. *South African Forestry Journal* **97**, 18-28.
- Hodgson, L.M. (1976c). Some aspects of flowering and reproductive behaviour in *Eucalyptus grandis* (Hill) Maiden at J.D.M. Keet Forest Research Station. 2. The fruit, seedlings, self fertility, selfing and inbreeding effects. *South African Forestry Journal* **98**, 32-43.
- Homes, W. B.K., Holmes, F. M., and Martin, H. A. (1982). Fossil *Eucalyptus* Remains from the Middle Miocene Chalk Mountain Formation, Warrumbungle Mountains, New South Wales. *Proc. Linn. Soc. NSW*. 106 (4), 299-310.
- Hopper, S.D., Coates, D.J. and Burbidge, A.H. (1978). Natural hybridization and morphometric relationships between three mallee eucalypts in the Fitzgerald River national park, W.A. *Australian Journal of Botany* **26**, 310-333.
- Hopper, S.D. and Moran, G.F. (1981). Bird pollination and the mating system of *Eucalyptus stoatei*. *Australian Journal of Botany* **29**, 625-638.
- Ireland, J.C. and Griffin, A.R. (1984). Observations on the pollination ecology of *Eucalyptus muellerana* Howitt in east Gippsland. *Victorian Naturalist* **101**, 207-211.
- Johnson, L.A.S. (1972). Evolution and classification in *Eucalyptus*. *Proceedings of the Linnean Society of New South Wales* **97**, 11-29.
- Johnson, L.A.S. (1976). Problems of species and genera in *Eucalyptus* (Myrtaceae). *Plant Systematics and Evolution* **125**, 155-167.
- Johnson, L.A.S. and Briggs, B.G. (1983). Myrtaceae In 'Flowering Plants in Australia.' (Ed. B.D. Morley and H.R. Toelken) pp. 175-185 (Rigby: Sydney.)
- Kapoor, M.L. and Sharma, V.K. (1984). Potentiality of genetic manipulation for increasing biomass production in *Eucalyptus*. *The Indian Forester* **110**, 814-819.
- Ladiges, P.Y. and Ashton, D.H. (1974). Variation in some central Victorian populations of *Eucalyptus viminalis* Labill. *Australian Journal of Botany* **22**, 81-102.
- Ladiges, P.Y. and Humphries, C.J. (1983). A cladistic study of *Arillastrum*, *Angophora* and *Eucalyptus* (Myrtaceae). *Biological Journal of the Linnean Society* **87**, 105-134.
- Ladiges, P.Y. and Humphries, C.J. (1986). Relationships in the stringybarks, *Eucalyptus* L'Herit. informal subgenus *Monocalyptus* series *Capitellatae* and *Olsenianae*: phylogenetic hypotheses, biogeography and classification. *Australian Journal of Botany* **34**, 603-32.
- Ladiges, P.Y., Humphries, C.J. and Brooker, M.I.H. (1983). Cladistic relationships and biogeographical patterns in the peppermint group of *Eucalyptus* (informal subseries *Amygdalinae*, subgenus *Monocalyptus*) and the description of a new species, *E. willisii*. *Australian Journal of Botany* **31**, 565-84.

- Marien, J.N. (1988). Action de basse temp ératures sur le pollen d' *Eucalyptus gunnii* In 'Annales de Recherches Sylvicoles 1987.' pp. 197-209 (AFOCEL: Paris.)
- Martin, B. (1989). The benefits of hybridization. How do you breed for them? In 'Breeding Tropical Trees: Population structure and genetic improvement strategies in clonal and seedling forestry. Proceedings of the IUFRO conference, Pattaya, Thailand, November 1988.' (Ed. G.L. Gibson, A.R. Griffin and A.C. Matheson) pp. 79-92 (Oxford, United Kingdom; Arlington, Virginia, USA: Oxford Forestry Institute; Winrock International.)
- Mergen, F., Lester, D.T., Furnival, G.M. and Burley, J. (1966). Discriminant analysis of *Eucalyptus cinerea* x *Eucalyptus maculosa* hybrids. *Silvae Genetica* **15**, 148-54.
- Meskimen, G. (1965). A tool for emasculating eucalypts. *Silvae Genetica* **13**, 57-58.
- Moncur, M.W. (1992). Effect of low temperature on floral induction of *Eucalyptus lansdowneana* F. Muell. & J. Brown subsp. *lansdowneana*. *Australian Journal of Botany* **40**, 157-67.
- Moncur, M.W. and Boland, D.J. (1989). Floral morphology of *Eucalyptus melliodora* A.Cunn. ex Schau. and flower architectural comparisons with other eucalypt species. *Australian Journal of Botany* **37**, 125-135.
- Moncur, M.W. and Hasan, O. (1994). Floral induction in *Eucalyptus nitens* (Deane and Maiden) Maiden. *Tree Physiology* **14**, 1303-1312.
- Moran, G.F. and Bell, J.C. (1983). *Eucalyptus* In 'Isozymes in Plant Genetics and Breeding.' (Ed. S.D. Tanksley and T.J. Orton.) pp. 423-441 (Elsevier: Amsterdam.)
- Moran, G.F., Bell, J.C. and Griffin, A.R. (1989). Reduction in levels of inbreeding in a seed orchard of *Eucalyptus regnans* F. Muell. compared with natural populations. *Silvae Genetica* **38**, 32-36.
- Nikles, D.G. and Griffin, A.R. (1992). Breeding hybrids of forest trees: definitions, theory, some practical examples, and guidelines on strategy with tropical acacias. *ACIAR Proc.* **37**, 101-109.
- Paton, D.M. (1978). *Eucalyptus* physiology 1. Photoperiod responses. *Australian Journal of Botany* **26**, 633-42.
- Peters, G.B., Lonie, J.S. and Moran, G.F. (1990). The breeding system, genetic diversity and pollen sterility in *Eucalyptus pulverulenta*, a rare species with small disjunct populations. *Australian Journal of Botany* **38**, 559-70.
- Pilipenka, F.S. (1969). 'Hybridisation of eucalypts in the USSR. Akademiya Nauk SSSR. Botanicheskii Institut. Trudy 6th series. Introduktsiya Rastenii Zelende Stroitel'stvd No. 9, 5-68.'
- Pole, M. (1989). Early Miocene floras from Central Ontago, New Zealand. *J. R. Soc. NZ* **19**, 121-127.
- Potts, B.M. (1988). 'Crossability patterns in *Eucalyptus*.' Research Working Group Number 1 Meeting Gympie, Queensland.)
- Potts, B.M. (1990). The response of eucalypt populations to a changing environment. *Tasforests* **2**, 179-193.
- Potts, B.M. and Cauvin, B. (1988). Inbreeding and interspecific hybridisation of *Eucalyptus* In 'Proceedings of the International Forestry Conference for the Australian Bicentenary.' (The Australian Forest Development Institute: Albury-Wodonga, Australia.)
- Potts, B.M. and Jackson, W.D. (1986). Evolutionary processes in the Tasmanian high altitude eucalypts In 'Flora and Fauna of Alpine Australasia. Ages and Origins.' (Ed. B.A. Barlow) pp. 511-527 (CSIRO: Melbourne, Australia.)
- Potts, B.M. and Marsden-Smedley, J.B. (1989). *In vitro* germination of *Eucalyptus* pollen: response to variation in boric acid and sucrose. *Australian Journal of Botany* **37**, 429-441.
- Potts, B.M. and Potts, W.C. (1986). Eucalypt breeding in France. *Australian Forestry* **49**, 210-218.
- Potts, B.M., Potts, W.C. and Cauvin, B. (1987). Inbreeding and interspecific hybridisation in *Eucalyptus gunnii*. *Silvae Genetica* **36**, 194-198.

- Potts, B.M. and Reid, J.B. (1985). Variation in the *Eucalyptus gunnii-archerii* complex. I. Variation in the adult phenotype. *Australian Journal of Botany* **33**, 337-359.
- Potts, B.M. and Reid, J.B. (1988). Hybridisation as a dispersal mechanism. *Evolution* **42**, 1245-1255.
- Potts, B.M. and Savva, M. (eds.) (1988). 'Self-incompatibility in *Eucalyptus*.' Pollination '88 (Plant Cell Biology Research Centre: University of Melbourne: Melbourne.)
- Potts, B.M. and Savva, M. (1989). The crossability of *Eucalyptus globulus* In 'Breeding Tropical Trees: Population structure and genetic improvement strategies in clonal and seedling forestry. Proceedings of the IUFRO conference, Pattaya, Thailand, November 1988.' (Ed. G.L. Gibson, A.R. Griffin and A.C. Matheson) pp. 420-422 (Oxford, United Kingdom; Arlington, Virginia, USA: Oxford Forestry Institute; Winrock International.)
- Potts, B.M., Volker, P.W. and Dungey, H.S. (1992) Barriers to the production of hybrids in *Eucalyptus*. In 'Mass Production Technology for Genetically Improved Fast Growing Forest Tree Species (AFOCEL-IUFRO Symposium 1992)', Bordeaux. (Association Foret Cellulose: Nangis - France). pp 193-204
- Prober, S.M., Tompkins, C., Moran, G.F. and Bell, J.C. (1990). The conservation genetics of *Eucalyptus paliformis* L. Johnson et Blaxell and *E. parvifolia* Cambage, two rare species from south-eastern Australia. *Australian Journal of Botany* **38**, 79-95.
- Pryor, L.D. (1957). Selecting and breeding for cold resistance in *Eucalyptus*. *Silvae Genetica* **6**, 98-109.
- Pryor, L.D. (1976). 'The biology of eucalypts.' (Edward Arnold: London.)
- Pryor, L.D. and Boden, R.W. (1962). Blowflies as pollinators in producing *Eucalyptus* seed. *Australian Journal of Science* **24**, 326.
- Pryor, L.D. and Johnson, L.A.S. (1971). 'A classification of the Eucalypts.' (Australian National University Press: Canberra.)
- Pryor, L.D. and Johnson, L.A.S. (1981). *Eucalyptus*, the universal Australian In 'Ecological Biogeography of Australia.' (Ed. A. Keast) pp. 499-536 (W. Junk: The Hague.)
- Pryor, L.D. and Knox, R.B. (1971). Operculum development and evolution in eucalypts. *Australian Journal of Botany* **19**, 143-171.
- Pryor, L.D. and Willing, R.R. (1974). The production of interspecific hybrids between incompatible pairs of eucalypts. *Combined Proceedings of the International Plant Propagation Society* **24**, 265-268.
- Ruggeri, C. (1960). Primo contributo alla conoscenza cariológica del genere *Eucalyptus* (Myrtaceae). *Publicazione* **IV**, 123-125.
- Rye, B.L. (1980). 'Chromosome numbers, reproductive biology and evolution in the Myrtaceae.' Unpublished Unpublished Thesis, University of West Australia, Perth.
- Sale, M.M., Potts, B.M., West, A.K. and Reid, J.B. (1993). Relationships within *Eucalyptus* using chloroplast DNA. *Australian Systematic Botany* **6**, 127-138.
- Savva, M., Potts, B.M. and Reid, J.B. (1988). The breeding system and gene flow in *Eucalyptus urnigera*. In 'Pollination '88.' (Ed. R.B. Knox, M.B. Sing and L.F. Troiani) pp. 176-182 (Plant Cell Biology Research Centre: University of Melbourne: Melbourne.)
- Sedgley, M. (1989). Ovule and seed development in *Eucalyptus woodwardii* Maiden (*Symphyomyrtus*). *Botanical Gazette* **150**, 271-280.
- Sedgley, M. and Griffin, A.R. (1989). 'Sexual reproduction of tree crops.' (Academic Press: London.)
- Sedgley, M., Hand, F.C., Smith, R.M. and Griffin, A.R. (1989). Pollen tube growth and early seed development in *Eucalyptus regnans* F. Muell. (Myrtaceae) in relation to ovule structure and preferential outcrossing. *Australian Journal of Botany* **37**, 397-411.
- Sedgley, M. and Smith, R.M. (1989). Pistil receptivity and pollen tube growth in relation to the breeding system of *Eucalyptus woodwardii* (*Symphyomyrtus*: Myrtaceae) d. *Ann. Bot.* **64**, 21-32.
- Sedgley, M.A.S., R.M. (1989). Pistil receptivity and pollen tube growth in relation to the breeding system of *Eucalyptus woodwardii* (*Symphyomyrtus*: Myrtaceae). *Annals of Botany* **64**, 21-32.

- Singh, G., Kershaw, A.P. and Clark, R. (1981). Quaternary vegetation and fire history in Australia. In 'Fire and the Australian biota.' (Ed. A.M. Gill, R.H. Groves and I.R. Noble) pp. 23-54 (Australian Academy of Science: Canberra.)
- Tibbits, W.N. (1988). Germination and morphology of progeny from controlled pollinations of *Eucalyptus nitens* (Deane and Maiden) Maiden. *Australian Journal of Botany* **36**, 677-691.
- Tibbits, W.N. (1989). Controlled pollination studies with shining gum (*Eucalyptus nitens* (Deane and Maiden) Maiden). *Forestry* **62**, 111-126.
- Tibbits, W.N. (1991) Flowering and seed yields in orchards of *Eucalyptus nitens* (D&M) Maiden. In 'Proceedings of the 9th Meeting of representative of the Research Working Group 1 of the Australian Forestry Council', Coonawarra. pp 79
- Vaillancourt, R. E., Potts, B. M., Manson, A., Eldridge, T. and Reid, J. B. (1995). Using RAPDs to detect QTLs in an interspecific F2 hybrid of *Eucalyptus*. In 'Eucalypt Plantations, Improving Fibre Yield and Quality' (eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond). pp. 430-433. Proc. CRCTHF-IUFRO Conf., Hobart, 19-24 Feb. (CRC for Temperate Hardwood Forestry: Hobart).
- van Loon, A.P. (1966). 'Investigations in regenerating the Tallowwood - Blue gum forest type.' Research note no. 19, Forestry Commission of N.S.W.
- van Wyk, G. (1977). Pollen handling, controlled pollination and grafting of *Eucalyptus grandis*. *South African Forestry Journal* **101**, 47-53.
- van Wyk, G. (1981a). Inbreeding effects on *Eucalyptus grandis* families in relation to degree of relatedness. *South African Forestry Journal* **116**, 60-63.
- van Wyk, G. (1981b). Pollen management for Eucalypts. In 'Pollen management handbook. Agriculture handbook no. 587.' (USDA Forest Service:
- Volker, P.B. and Gore, P.G. (1988) Flowering patterns in a multi-provenance seed orchard of *Eucalyptus globulus*. In 'Proceedings of the Research Working Group No. 1 of the Australian Forestry Council, Forest Genetics.', Gympie, November 1988. (Australian Forestry Council: Canberra.).
- Volker, P.W. (1995). Evaluation of *Eucalyptus nitens* x *globulus* for commercial forestry. In 'Eucalypt Plantations, Improving Fibre Yield and Quality' (eds. B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond). pp. 222-225. Proc. CRCTHF-IUFRO Conf., Hobart, 19-24 Feb. (CRC for Temperate Hardwood Forestry: Hobart).
- Wallace, H. (1991) Dispersal of seeds of *Eucalyptus toelliana* F. Muell. by the stingless bee *Trigona Carbonaria* Smith. In 'Aust. Ent. Soc. 22nd A.G.M. & Scientific Conference', Melbourne 14-19 July 1991.
- Whitham, T., Morrow, P. and Potts, B.M. (1991). 'The response of insect pests to eucalypt hybrids.' Research Working Group No. 1 of the Australian Forestry Council, Forest Genetics. Proceedings of Eleventh Meeting, Coonawarra; March 1991. (Australian Forestry Council: Canberra.)
- Wilcox, M.D. (1982). Anothcyanin polymorphism in seedlings of *Eucalyptus fastigiata* Deane & Maid. *Australian Journal of Botany* **30**, 501-509.
- Wiltshire, R.J.E., Potts, B.M. and Reid, J.B. (1991). A pedomorphocline in *Eucalyptus*: I. Variation in seedling morphology in the *E. risdonii/tenuiramis* complex. *Australian Journal of Botany* **39**, 545-66.